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(54) Title: PEPTIDE WHICH ABROGATES TNF AND/OR LPS TOXICITY

VRSSSRTPSD10KPVAHVVANP20QAEGQLQWLN30RRA

NALLANG40VELRDNQLVV50PSEGLYLIYS60QVLFKGQGCP70STHVLL

THTI80SRIAVSYQTK90VNLLSAIKSP100CQRETREGAE110AKPWYEPI

YL120GGVFQLEKGD130RLSAEINRPD140YLDFAESGQV150YFGIIAL157

(57) Abstract

The present invention provides peptides which have the ability to abrogate TNF toxicity and/or LPS toxicity. The present invention further relates to compositions including these peptides as the active ingredient and methods of anti-inflammatory treatment involving the administration of this composition. The peptides of the present invention are based primarily on residue 1 to 26 of human TNF.

### PEPTIDE WHICH ABROGATES TNF AND/OR LPS TOXICITY

#### Field of the Invention

The present invention relates to a group of peptides which have the ability to abrogate TNF toxicity and/or LPS

5 toxicity. The present invention further relates to compositions including this peptide as the active ingredient and methods of anti-inflammatory treatment involving the administration of this composition.

#### Background of the Invention

10 Many of the clinical features of septicemic shock induced by Gram-negative bacteria which have lipopolysaccharide (LPS) in their cell walls may be reproduced in animals by the administration of LPS. This induces prompt severe metabolic and physiological changes

15 which can lead to death. Associated with the injection of LPS is the extensive production of tumour necrosis factor alpha (TNF). Many of the effects of LPS injection or indeed of Gram-negative bacteria can be reproduced by TNF. Thus, mice injected with recombinant human TNF

20 develop piloerection of the hair (ruffling), diarrhoea, a withdrawn, unkempt appearance and die if sufficient amounts are given. Rats treated with TNF become hypotensive, tachypneic and die of sudden respiratory arrest (Tracey et al., 1986 Science 234, 470). Severe

25 acidosis, marked haemoconcentration and biphasic changes in blood glucose concentration were also observed. Histopathology revealed severe leukostatsis in the lungs, haemorraghic necrosis in the adrenals, pancreas and other organs and tubular necrosis of the kidneys. All these

30 changes were prevented if the animals were pretreated with a neutralizing monoclonal antibody against TNF.

The massive accumulation of neutrophils in the lungs of TNF-tr ated animals reflects the activation of neutrophils by TNF. TNF causes neutrophil degranulation,

35 respiratory burst, enhanced antimicrobiocidal and

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anti-tumour activity (Klebanoff et al., 1986 J. Immunol.
136, 4220; Tsujimoto et al., 1986 Biochem Biophys Res
Commun 137, 1094). Endothelial cells are also an
important target for the expression of TNF toxicity. TNF
5  diminishes the anticoagulant potential of the endothelium,
inducing procoagulant activity and down regulation of the
expression of thrombomodulin (Stern and Nawroth, 1986 J
Exp Med 163, 740).

TNF, a product of activated macrophages produced in
10  response to infection and malignancy, was first identified
as a serum factor in LPS treated mice which caused the
haemorraghic necrosis of transplantable tumours in murine
models and was cytoxoic for tumour cells in culture
(Carswell et al., 1975 PNAS 72, 3666; Helson et al., 1975
15  Nature 258, 731). Cachexia is a common symptom of
advanced malignancy and severe infection. It is
characterised by abnormal lipid metabolism with
hypertriglyceridemia, abnormal protein and glucose
metabolism and body wasting. Chronic administration of
20  TNF (also known as cachectin in the early literature) to
mice causes anorexia, weight loss and depletion of body
lipid and protein within 7 to 10 days (Cerami et al., 1985
Immunol Lett 11, 173, Fong et al., 1989 J Exp Med 170,
1627). These effects were reduced by concurrent
25  administration of antibodies against TNF. Although TNF
has been measured in the serum of patients with cancer and
chronic disease associated with cachexia the results are
inconclusive since large differences in TNF levels have
been reported. These may be due to the short half-life of
30  TNF (6 minutes), differences in TNF serum binding protein,
or true differences in TNF levels in chronic disease
states.

TNFα, as a mediator of inflammation, has been
implicated in the pathology of other diseases apart from
35  toxic shock and cancer-related cachexia. TNF has been

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measured in synovial fluid in patients with both
rheumatoid and reactive arthritis and in the serum of
patients with rheumatoid arthritis (Saxne et al., 1988
Arthrit. Rheumat. 31, 1041). Raised levels of TNF have
5 been detected in renal transplant patients during acute
rejection episodes (Maury and Teppo 1987 J. Exp Med 166,
1132). In animals TNF has been shown to be involved in
the pathogenesis of graft versus host disease in skin and
gut following allogeneic marrow transplantation.
10 Administration of a rabbit anti-murine TNF was
demonstrated to prevent the histological changes
associated with graft versus host disease and reduced
mortality (Piquet et al., 1987 J Exp Med 166, 1280).

TNF has also been shown to contribute significantly
15 to the pathology of malaria (Clark et al., 1987; Am. J.
Pathol. 129: 192-199). Further, elevated serum levels of
TNF have been reported in malaria patients (Scuderi
et al., 1986; Lancet 2: 1364-1365). TNF may also
contribute to the brain pathology and consequent dementia
20 observed in late stage HIV infections (Grimaldi et al Ann
Nevrol 29 : 21)

The peptides encompassed in the present invention do
not necessarily interfere directly with the bio-synthetic
mechanisms of the disease-causing component. As will be
25 described below in the experimental data the mechanism
behind the alleviating effect of the peptides is to be
found in the modulation of the different cytokines
produced by activated cells belonging to the cell-lines
encompassing the immune defence. This modulation of
30 cytokines is not limited to TNF but may also be valid for
the whole range of interleukins, from interleukin-1 to
interleukin 10. LPS, a known component of bacteria
important in inducing major inflammatory response was used
as a model. LPS binds to receptors on neutrophils,
35 monocytes, endothelial cells and machrophages, which

consequently become activated and start production of IL-1 and TNF and other cytokines, thus starting the inflammatory cascade. One parameter used to measure the effect of LPS is the concentration of blood glucose, which

5 will normally decrease on exposure to TNF or LPS.

LPS normally combines with LPS-Binding-Protein (LBP) and exerts its dramatic effect through the CD14 receptor. The activation of the CD14 molecule by LPS results in TNF production by leucocytes. It is believed that the

10 peptides of the present invention which abrogate LPS toxicity may exert their effect by interacting with the CD14 molecule and thus inhibit LPS binding.

The peptides identified by the present inventors which have the ability to abrogate TNF and/or LPS toxicity

15 resemble peptide sequences found in the amino terminal of TNFα . Other investigators have also considered this area of the TNFα molecule but with little success in obtaining biologically active peptides.

In this regard attention is drawn to Canadian patent

20 application Nos 2005052 and 2005056 in the name of BASF AG. Both these applications claim a wide range of peptide sequences and, by selecting appropriate alternatives it can be seen that application No 2005052 is directed toward the peptide sequence 7-42 of TNFα whilst application No

25 2005056 is directed toward amino acid sequence 1 to 24 of TNFα . Whilst each of these applications claim a broad range of peptide sequences it is noted that there is no indication as to what, if any, biological activity the claimed peptides may possess. Indeed there is no

30 demonstration that any of the produced peptide have any biological activity. In contrast, the present inventors have produced a range of peptides which have specific activities in that they abrogate TNF and/or LPS toxicity.

Summary of the Invention

35 In a first aspect the present invention consists in a linear or cyclic peptide of the general formula:-

$$X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9$$

in which

$X_1$ is null, Cys or $R_1$

$X_2$ is null, Cys, $R_1$ or $A_1-A_2-A_3-A_4-A_5$

5       in which   $A_1$ is Val or Ile or Leu or Met or His

                    $A_2$ is Arg or Cys or His

                    $A_3$ is Ser or Thr or Ala

                    $A_4$ is Ser or Thr or Ala

                    $A_5$ is Ser or Thr or Ala

10     $X_3$ is Cys, $R_1$ or $A_6-A_7$

       in which   $A_6$ is Arg or Cys or His or Absent

                    $A_7$ is Thr or Ser or Ala

$X_4$ is Cys, $R_1$ or $A_8-A_9$

       in which   $A_8$ is Pro or an Nα-alkylamino acid

15                      $A_9$ is Ser or Thr or Ala

$X_5$ is Cys, $R_1$ or $A_{10}$

       in which   $A_{10}$ is Asp or Ala or Cys or Glu or Gly
or Arg or His

$X_6$ is Cys, $R_2$ or $A_{11}-A_{12}-A_{13}$

20     in which   $A_{11}$ is absent or Cys or Arg or His or
Asp or Glu

                    $A_{12}$ is Pro or an Nα-alkylamino acid

                    $A_{13}$ is Val or Ile or Phe or Tyr or Trp
or His or Leu or His or Met

25     $X_7$ is null, Cys, $R_2$ or $A_{14}-A_{15}$

       in which   $A_{14}$ is Ala or Val or Gly or Ile or Phe
or Trp or Tyr or Leu or His or Met

                    $A_{15}$ is absent or His or Arg or Glu or
Asa or Ala or Lys or Asp or Phe or Tyr or

30                      Trp or Glu or Gln or Ser or Thr or Gly

$X_8$ is null, Cys, $R_2$, $A_{16}$, $A_{16}-A_{17}$, $A_{16}-A_{17}-A_{18}$ or
$A_{16}-A_{17}-A_{18}-A_{19}-A_{20}-A_{21}-A_{22}-A_{23}-A_{24}-A_{25}-A_{26}$

       in which   $A_{16}$ is Val or Ile or Leu or Met or His

                    $A_{17}$ is Val or Ile or Leu or Met or His

35                    $A_{18}$ is Ala or Gly

$A_{19}$ is Asp or Glu

$A_{20}$ is Pro or an N$\alpha$-alkylamino acid

$A_{21}$ is Gln or Asn

$A_{22}$ is Ala or Gly ,

$A_{23}$ is Glu or Asp

$A_{24}$ is Gly or Ala

$A_{25}$ is Gln or Asn

$A_{26}$ is Leu or Ile or Val or Met or His

$X_9$ is null, Cys or $R_2$

$R_1$ is R-CO, where R is H, straight, branched or cyclic alkyl up to C20, optionally containing double bonds and/or substituted with halogen, nitro, amino, hydroxy, sulfo, phospho or carboxyl groups (which may be substituted themselves), or aralkyl or aryl optionally substituted as listed for the alkyl and further including alkyl, or $R_1$ is glycosyl, nucleosyl, lipoyl or $R_1$ is an L- or D-$\alpha$ amino acid or an oligomer thereof consisting of up to 5 residues $R_1$ is absent when the amino acid adjacent is a desamino-derivative.

$R_2$ is

-$NR_{12}R_{13}$, wherein $R_{12}$ and $R_{13}$ are independently H, straight, branched or cyclic alkyl, aralkyl or aryl optionally substituted as defined for $R_1$ or N-glycosyl or N-lipoyl

-$OR_{14}$, where $R_{14}$ is H, straight, branched or cyclic alkyl, aralkyl or aryl, optionally substituted as defined for $R_1$

-O-glycosyl, -O-lipoyl or

- an L- or D-$\alpha$-amino acid or an oligomer thereof consisting of up to 5 residues

or $R_2$ is absent, when the adjacent amino acid is a decarboxy derivative of cysteine or a homologue thereof or the peptide is in a N-C cyclic form.

with the proviso that:

when $X_6$ is Cys or $R_2$ then $X_5$ is $A_{10}$, $X_4$ is $A_8-A_9$,
   $X_3$ is $A_6-A_7$ and $X_2$ is $A_1-A_2-A_3-A_4-A_5$
when $X_5$ is Cys or $R_1$ then $X_6$ is $A_{11}-A_{12}-A_{13}$, $X_7$ is
   $A_{14}-A_{15}$, $X_8$ is $A_{16}-A_{17}-A_{18}$ and $A_{11}$ is absent
5  when $X_4$ is Cys or $R_1$ then $X_5$ is $A_{10}$, $X_6$ is
   $A_{11}-A_{12}-A_{13}$, $X_7$ is $A_{14}-A_{15}$ and $X_8$ is
   $A_{16}-A_{17}-A_{18}$
when $X_2$ is $A_1-A_2-A_3-A_4-A_5$ then $X_8$ is not $A_{16}$
when $X_1$ is null, $X_2$ is Cys or $R_1$, $X_3$ is $A_6-A_7$, $X_4$ is
10  $A_8-A_9$, $X_5$ is $A_{10}$, $X_6$ is $A_{11}-A_{12}-A_{13}$, $X_7$ is
   $A_{14}-A_{15}$ and $X_8$ is $A_{16}$ then $A_{16}$ is not D-His.
$X_1$ is always and only null when $X_2$ is $R_1$, Lys or Null
$X_2$ is always and only null when $X_3$ is Cys or $R_1$
$X_3$ is always and only null when $X_6$ is Cys or $R_2$
15  $X_7$ is always and only null when $X_7$ is Cys, $R_2$ or Null
$X_8$ is always and only null when $X_8$ is Cys, $R_2$ or Null
$X_9$ is always and only null when $X_8$ is Cys, $R_2$ or Null
when $X_1$ and $R_2$ are null, $X_3$ is $R_1$, $X_4$ is
20  $A_8-A_9$, $X_5$ is $A_{10}$, $X_6$ is $A_{11}-A_{12}-A_{13}$, $X_7$
   is $A_{14}-A_{15}$, $X_8$ is $R_2$ and $A_{14}$ is Ala and $A_{15}$ is
   absent then $R_1$ is acetyl and $R_2$ is $NH_2$.

The amino acids may be D or L isomers, however
generally the peptide will primarily consist of L-amino
25  acids.

In a second aspect the present invention consists in
a pharmaceutical composition for use in treating subjects
suffering from toxic effects of TNF and/or LPS, the
composition comprising a therapeutically effective amount
30  of a peptide of the first aspect of the present invention
and a pharmaceutically acceptable sterile carrier.

In a third aspect the present invention consists in a
method of treating a subject suffering from the toxic
effects of TNF and/or LPS, the method comprising
35  administering to the subject a therapeutically effective
amount of the composition of the second aspect of the
present invention.

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In a preferred embodiment of the present invention
$X_1$ is H, $X_2$ is $A_1$-$A_2$-$A_3$-$A_4$-$A_5$, $X_3$ is
$A_6$-$A_7$, $X_4$ is $A_8$-$A_9$, $X_5$ is $A_{10}$, $X_6$ is
$A_{11}$-$A_{12}$-$A_{13}$, $X_7$ is $A_{14}$-$A_{15}$, $X_8$ is
5   $A_{16}$-$A_{17}$-$A_{18}$ and $X_9$ is OH.

In a further preferred embodiment of the present
invention $X_1$ is null, $X_2$ is H or Ac, $X_3$ is
$A_6$-$A_7$, $X_4$ is $A_8$-$A_9$, $X_5$ is $A_{10}$, $X_6$ is
$A_{11}$-$A_{12}$-$A_{13}$, $X_7$ is $A_{14}$-$A_{15}$, $X_8$ is
10  $A_{16}$-$A_{17}$-$A_{18}$ and $X_9$ is OH or $NH_2$.

In a further preferred embodiment of the present
invention $X_1$ is H, $X_2$ is $A_1$-$A_2$-$A_3$-$A_4$-$A_5$,
$X_3$ is $A_6$-$A_7$, $X_4$ is $A_8$-$A_9$, $X_5$ is $A_{10}$, $X_6$
is OH and $X_6$, $X_7$ and $X_8$ are null.

15  In a further preferred embodiment of the present
invention the peptide is selected from the group
consisting of:-

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala
-His-Val-Val-Ala;

20  Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val-Val-Ala;

Arg-Thr-Pro-Ser-Ala-Lys-Pro-Val-Ala-His-Val-Val-Ala;

Arg-Thr-Pro-Ser-Lys-Asp-Pro-Val-Ala-His-Val-Val-Ala;

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala
-Arg-Val-Val-Ala;

25  Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala
-Gln-Val-Val-Ala;

Ac-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val-NH2;

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-Ala-Val;

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-Lys-Val;

30  Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val-Val;

Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val;

Pro-Ser-Asp-Lys-Pro-Val-Ala-His;

Pro-Ser-Asp-Lys-Pro-Val;

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-

35  Val-His-Val-Val-Ala;

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Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val-Val-Ala-Asn
-Pro-Gln-Ala-Glu-Gly-Gln-Leu;
Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp;
Ac-Pro-Ser-Asp-Lys-Pro-Val-Ala-NH2;
5      Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-Asp-Val;
Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-
Ala-His-Val-Val-Ala-Asn-Pro-Gln-Ala-Glu-Gly-Gln-Leu;
Asp-Lys-Pro-Val-Ala-His-Val-Val-Ala;
Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val;
10     Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val-Val-Ala;
Pro-Sir-Asp-Lys-Pro-Val-Ala-His-Val-Val-Ala;
Pro-Val-Ala-His-Val-Val-Ala;   and
Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Val-His-Val.

The composition and method of the present invention
15 would be expected to be useful as an anti-inflammatory
agent in a wide range of disease states including toxic
shock, adult respiratory distress syndrome,
hypersensitivity pneumonitis, systemic lupus
erythromatosis, cystic fibrosis, asthma, bronchitis, drug
20 withdrawal, schistosomiasis, sepsis, rheumatoid arthritis,
acquired immuno-deficiency syndrome, multiple sclerosis,
leperosy, malaria, systemic vasculitis, bacterial
meningitis, cachexia, dermatitis, psoriasis, diabetes,
neuropathy associated with infection or autoimmune
25 disease, ischemia/reperfusion injury, encephalitis,
Guillame Barre Syndrome, atherosclerosis, chronic fatigue
syndrome, TB, other viral and parasitic diseases, OKT3
therapy, and would be expected to be useful in conjunction
with radiation therapy, chemotherapy and transplantation,
30 to ameliorate the toxic effects of such treatments or
procedures.

As the peptide of the present invention suppresses
activation of neutrophils the composition and method of
the present invention may also be useful in the treatment
35 of diseases with an underlying element of local, systemic,
acute or chronic inflammation.  In gen ral, it is believed

the composition and method of the present invention will be useful in treatment of any systemic or local infection leading to inflammation.

The peptides of the present invention may also be
5 administered in cancer therapy in conjunction with cytotoxic drugs which may potentiate the toxic effects of TNFα (Watanabe et al., 1988; Immunopharmacol. Immunotoxicol. 10: 117-127) such as vinblastin, acyclovir, interferon alpha, cyclosporin A, IL-2, actinomycin D,
10 adriamycin, mitomycin C, AZT, cytosine arabinoside, daunororubin, cis-platin, vincristine, 5-flurouracil and bleomycin; in cancer patients undergoing radiation therapy; and in AIDS patients (or others suffering from viral infection such as viral meningitis, hepatitis,
15 herpes, green monkey virus etc.) and in patients receiving immunostimulants such as thymopentin and muramyl peptides or cytokines such as IL-2 and GM-CSF. In this use peptides of the present invention will serve to abrogate the deleterious effects of TNFα

20 It will be appreciated by those skilled in the art that a number of modifications may be made to the peptide of the present invention without deleteriously effecting the biological activity of the peptide. This may be achieved by various changes, such as insertions, deletions
25 and substitutions (e.g., sulfation, phosphorylation, nitration, halogenation), either conservative or non-conservative (e.g., W-amino acids, desamino acids) in the peptide sequence where such changes do not substantially altering the overall biological activity of
30 the peptide. By conservative substitutions the intended combinations are:-

G, A; V, I, L, M; D, E; N, Q; S, T; K, R, H; F, Y, W, H; and P, Nα-alkylamino acids.

It may also be possible to add various groups to the
35 peptide of the present invention to confer advantages such as increased potency or extended half-life in vivo,

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without substantially altering the overall biological
activity of the peptide.

The term peptide is to be understood to embrace
peptide bond replacements and/or peptide mimetics, i.e.
5    pseudopeptides, as recognised in the art (see for example:
Proceedings of the 20th European Peptide Symposium, edt.
G. Jung. E. Bayer, pp. 289-336, and references therein),
as well as salts and pharmaceutical preparations and/or
formulations which render the bioactive peptide(s)
10   particularly suitable for oral, topical, nasal spray,
ocular pulmonary, I.V., subcutaneous, as the case may be,
delivery.  Such salts, formulations, amino acid
replacements and pseudopeptide structures may be necessary
and desirable to enhance the  stability, formulation,
15   deliverability (e.g., slow release, prodrugs), or to
improve the economy of production, and they are
acceptable, provided they do not negatively affect the
required biological activity of the peptide.

Apart from substitutions, three particular forms of
20   peptide mimetic and/or analogue structures of particular
relevance when designating bioactive peptides, which have
to bind to a receptor while risking the degradation by
proteinases and peptidases in the blood, tissues and
elsewhere, may be mentioned specifically, illustrated by
25   the following examples: Firstly, the inversion of backbone
chiral centres leading to D-amino acid residue structures
may, particularly at the N-terminus, lead to enhanced
stability for proteolytical degradation while not
impairing activity.  An example is given in the paper
30   "Tritriated D-ala$^1$-Peptide T Binding", Smith, C.S. et
al, Drug Development Res. 15, pp. 371-379 (1988).
Secondly, cyclic structure for stability, such as N to C
interchain imides and lactames (Ede et al in Smith and
Rivier (Eds) "Peptides: Chemistry and Biology", Escom,
35   Leiden (1991), p268-270), and sometimes also receptor
binding may be enhanced by forming cyclic analogues.  An

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example of this is given in "Confirmationally restricted
thymopentin-like compounds", U.S. pat. 4,457,489 (1985),
Goldstein, G. et al.  Finally, the introduction of
ketomethylene, methylsulfide or retroinverse bonds to
5  replace peptide bonds, i.e. the interchange of the CO and
NH moieties may both greatly enhance stability and
potency.  An example of the latter type is given in the
paper "Biologically active retroinverso analogues of
thymopentin", Sisto A. et al in Rivier, J.E. and Marshall,
10  G.R. (eds.) "Peptides, Chemistry, Structure and Biology",
Escom, Leiden (1990), p.722-773.

The peptides of the invention can be synthesized by
various methods which are known in principle, namely by
chemical coupling methods (cf. Wunsch, E.: "Methoden der
15  organischen Chemie", Volume 15, Band 1 + 2, Synthese von
Peptiden, Thieme Verlag, Stuttgart (1974), and Barrany, G.;
Merrifield, R.B: "The Peptides", eds. E. Gross,
J. Meienhofer., Volume 2, Chapter 1, pp. 1-284, Academic
Press (1980)), or by enzymatic coupling methods
20  (cf. Widmer, F., Johansen, J.T., Carlsberg Res. Commun.,
Volume 44, pp. 37-46 (1979), and Kullmann, W.: "Enzymatic
Peptide Synthesis", CRC Press Inc., Boca Raton, Florida
(1987), and Widmer, F., Johansen, J.T. in "Synthetic
Peptides in Biology and Medicine:, eds., Alitalo, K.,
25  Partanen, P., Vatieri, A., pp. 79-86, Elsevier, Amsterdam
(1985)), or by a combination of chemical and enzymatic
methods if this is advantageous for the process design and
economy.

It will be seen that one of the alternatives embraced
30  in the general formula set out above is for a cysteine
residue to be positioned at both the amino and carboxy
terminals of the peptide.  This will enable the cylisation
of the peptide by the formation of di-sulphide bond.

It is intended that such modifications to the peptide
35  of the present invention which do not result in a decrease
in biological activity are within the scope of the present
invention.

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As would be recognized by those skilled in the art
there are numerous examples to illustrate the ability of
anti-idiotypic (anti-Ids) antibodies to an antigen to
function like that antigen in its interaction with animal
5    cells and components of cells.  Thus, anti-Ids to a
peptide hormone antigen can have hormone-like activity and
interact specifically with the receptors to the hormone.
Conversely, anti-Ids to a receptor can interact
specifically with a mediator in the same way as the
10   receptor does.  (For a review of these properties see:
Gaulton, G.N. and Greane, M.I. 1986.  Idiotypic mimicry of
biological receptors, Ann. Rev. Immunol. 4, 253-280;
Sege, K and Peterson, P.A., 1978.  Use of anti-iodiotypic
antibodies as cell surface receptor probes.  Proc. Natl.
15   Acad. Sci. U.S.A. 75, 2443-2447).

As might be expected from this functional similarity
of anti-Id and antigen, anti-Ids bearing the internal
image of an antigen can induce immunity to such an
antigen.  (This nexus is reviewed in Hiernaux, J.R. 1988.
20   Idiotypic vaccines and infectious diseases.  Infect.
Immun. 56, 1407-1413.)

As will be appreciated by persons skilled in the art
from the disclosure of this application it will be
possible to produce anti-idiotypic antibodies to the
25   peptide of the present invention which will have similar
biological activity.  It is intended that such
anti-idiotypic antibodies are included within the scope of
the present invention.

Accordingly, in a fourth aspect the present invention
30   consists in an anti-idiotypic antibody to the peptide of
the first aspect of the present invention, the
anti-idiotypic antibody being capable of abrogating TNF
and/or LPS toxicity.

The individual specificity of antibodies resides in
35   the structures of the peptide loops making up the
Complementary Determining Regions (CDRs) of the variable

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domains of the antibodies. Since in general, the amino
acid sequences of the CDR peptide loops of an anti-Id are
not identical to or even similar to the amino acid
sequence of the peptide antigen from which it was
5   originally derived, it follows that peptides whose amino
acid sequence is quite dissimilar, in certain contexts can
take up a very similar three-dimensional structure. The
concept of this type of peptide, termed a "functionally
equivalent sequence" or mimotope by Geyson is familiar to
10  those expert in the field. (Geyson, H.M. et al 1987.
Strategies for epitope analysis using peptide synthesis.
J. Immun. Methods. 102, 259-274).

    Moreover, the three-dimensional structure and
function of the biologically active peptides can be
15  simulated by other compounds, some not even peptidic in
nature, but which mimic the activity of such peptides.
This field of science is summarised in a review by
Goodman, M. (1990). (Synthesis, spectroscopy and computer
simulations in peptide research. Proc. 11th American
20  Peptide Symposium published in Peptides-Chemistry.
Structure and Biology pp 3-29. Ed Rivier, J.E. and
Marshall, G.R. Publisher ESCOM.)

    As will be recognized by those skilled in the art,
armed with the disclosure of this application, it will be
25  possible to produce peptide and non-peptide compounds
having the same three-dimensional structure as the peptide
of the present invention. These "functionally equivalent
structures" or "peptide mimics" will react with antibodies
raised against the peptide of the present invention and
30  may also be capable of abrogating TNF toxicity. It is
intended that such "peptide mimics" are included within
the scope of the present invention.

    Accordingly, in a fifth aspect the present invention
consists in a compound the three-dimensional structure of
35  which is similar as a pharmacophore to the three-
dimensional structure of the peptide of the first aspect

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of the present invention, the compound being characterized
in that it reacts with antibodies raised against the
peptide of the first aspect of the present invention and
that the compound is capable of abrogating TNF and/or LPS
5   toxicity.

More detail regarding pharmacophores can be found in
Bolin et al. p 150, Polinsky et al. p 287, and Smith et
al. p 485 in Smith and Rivier (Eds) "Peptides: Chemistry
and Biology", Escom, Leiden (1991).

10  Detailed Description of the Invention

In order that the nature of the present invention may
be more clearly understood, the preferred forms thereof
will now be described with reference to the following
example and accompanying Figures and Tables in which:

15        Fig. 1 shows the amino acid sequence of human TNFα ;

Fig. 2: Effect of TNF (▱) and TNF+ Peptide 1 (◆) on
blood glucose levels in malaria primed mice-Peptide 1
abrogates TNF induced hypoglycaemia in malaria primed mice.

Fig. 3: Effect of Peptide 1 on TNF-induced tumour
20  regression.

Fig. 4: Effect of Peptide 1 (●), peptide 308 (▼),
peptide 309 (▄), peptide 305 (▨) and peptide 302 (O) on
binding of radiolabelled TNF to TNF receptors on WEH1-164
tumour cells - Peptide 1 does not inhibit binding of TNF
25  to tumour cells.

Fig. 5: Plasma reactive nitrogen intermediate levels
in TNF± Peptide 1 treated malaria primed mice - this shows
that induction of RNI by TNF is inhibited by treatment
with Peptide 1.

30        Fig. 6 shows the effect on blood glucose levels in
mice treated with PBS (▱);   TNF alone (◆);
TNF + Peptide 1 (▄) and TNF + Peptide 2 (O).

Fig. 7 shows the effect of Peptide 1 on TNF-induced
decrease in blood glucose levels in mice administered with
35  200μg TNF.

Fig. 8 shows the effect of Peptide 1 on TNF-induced decrease in blood glucose levels in ascites tumour-bearing mice.

Fig. 9 shows the effect of Peptide 1 on TNF-induced
5  weight loss in ascites tumour-bearing mice.

Fig. 10 shows the effect of peptides on LPS toxicity in Meth A ascites tumour-bearing mice (10 animals per group scored positive if 7 or more survive);

Fig. 11 shows the effect of peptides on LPS toxicity
10  in Meth A ascites tumour-bearing mice (10 animals per group scored positive if 7 or more survive);

Fig. 12 shows the effect of peptides on TNF toxicity in Meth A ascites tumour-bearing mice (each group contains 20 animals:  scored positive if 7 or more survived);

15  Fig. 13 shows the effect of peptides on TNF toxicity in Meth A ascites tumour-bearing mice (each group contains 20 animals:  scored positive if 10 or more survived);

Fig. 14 shows effect of peptides on TNF toxicity in D-galactosamine sensitized mice (each group contains 10
20  animals:  scored positive if 6 or more survive).

Fig. 15 shows the effect of peptides on direct induction of chemiluminescence by TNF on human neutrophils;

Fig. 16 shows inhibition of TNF priming of human neutrophils by Peptide 21;

25  Fig. 17 shows inhibition of TNF priming of human neutrophils by Peptide 19;

Fig. 18 shows inhibition of LPS stimulation of neutrophils by Peptide 19;

Fig. 19 shows dose-dependent effects of Peptide 9 on
30  TNF-induced chemiluminescence;

Fig. 20 shows effect of peptide 2 on human TNF priming of human neutrophils;

Fig. 21 shows inhibition of LPS-induced chemiluminescence response of human neutrophils by Peptide
35  21; and

Fig. 22 shows inhibition of TNF priming of human neutrophils by Peptide 21.

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## Production of Peptides

### Synthesis of Peptides Using the FMOC-Strategy

Peptides (1-6, 9-18, 22-25, 27-29, 35, 36, 39, 40
Table 3) were synthesized on the continuous flow system as
5  provided by the Milligen synthesizer Model 9050 using the
standard Fmoc-polyamide method of solid phase peptide
synthesis (Atherton et al, 1978, J.Chem. Soc. Chem.
Commun., 13, 537-539).

For peptides with free carboxyl at the C-terminus,
10  the solid resin used was PepSyn KA which is a
polydimethylacrylamide gel on Kieselguhr support with
4-hydroxymethylphenoxyacetic acid as the functionalised
linker (Atherton et al., 1975, J.Am.Chem.Soc 97,
6584-6585). The carboxy terminal amino acid was attached
15  to the solid support by a DCC/DMAP-mediated
symmetrical-anhydride esterification.

For peptides with carboxamides at the C-terminus, the
solid resin used was Fmoc-PepSyn L Am which is analogous
polyamides resin with a Rink linker,
20  p-[(R,S)-α[1-(9H-fluoren-9-yl)-methoxyformamido]-2,
4-dimethoxybenzyl]-phenoxyacetic acid (Bernatowicz et al,
1989, Tet.Lett. 30, 4645). The synthesis starts by
removing the Fmoc-group with an initial piperidine wash
and incorporation of the first amino acid is carried out
25  by the usual peptide coupling procedure.

The Fmoc strategy was also carried out in the stirred
cell system in synthesis of peptides (33,34,37,38) where
the Wang resin replaced the Pepsyn KA.

All Fmoc-groups during synthesis were removed by 20%
30  piperidine/DMF and peptide bonds were formed either of the
following methods except as indicated in Table 1:

1.  Pentafluorophenyl active esters. The starting
materials are already in the active ester form.

2.  Hydroxybenzotriazol esters. These are formed in situ
35  either using Castro's reagent, BOP/NMM/HOBt (Fournier et
al, 1989, Int.J.Peptide Protein Res., 33, 133-139) or

using Knorr's reagent, HBTU/NMM/HOBt (Knorr et al, 1989, Tet.Lett., 30, 1927).

Side chain protection chosen for the amino acids was removed concomitantly during cleavage with the exception of Acm on cysteine which was left on after synthesis. Intramolecular disulphide bridges where needed are then formed by treating the Acm protected peptide with iodine/methanol at high dilution.

TABLE 1

| Amino Acid | Protecting Group | Coupling Method |
|---|---|---|
| Arg | Pmc | HOBt or OPfp |
| Asp | OBut | HOBt or OPfp |
| Cys | Acm | HOBt or OPfp |
| Glu | OBut | HOBt or OPfp |
| His | Boc or Trt | HOBt or OPfp |
| Lys | But | HOBt or OPfp |
| Ser | But | HOBt only |
| Thr | But | HOBt only |
| Tyr | But | HOBt or OPfp |
| Asn | none | OPfp only |
| Gln | none | OPfp only |

Cleavage Conditions

Peptides were cleaved from the PepSyn KA and PepSyn K Am using 5% water and 95% TFA where Arg(Pmc) is not present. Where Arg(Pmc) is present a mixture of 5% thioanisole in TFA is used. The cleavage typically took 3 h at room temperature with stirring. Thioanisole was removed by washing with ether or ethyl acetate and the peptide was extracted into an aqueous fraction. Up to 30% acetonitrile was used in some cases to aid dissolution. Lyophilization of the aqueous/acetonitrile extract gave the crude peptide.

Peptides from the Wang resin were cleaved using 5% phenol, 5% ethanedithiol and 90% TFA for 16 h at ambient temperature with stirring. Thioanisole was removed by

washing with ether or ethyl acetate and the peptide was
extracted into an aqueous fraction.  Up to 30%
acetonitrile was used in some cases to aid dissolution.
Lyophilization of the aqueous/acetonitrile extract gave

5  the crude peptide.

Peptides from the Wang resin were cleaved using 5%
phenol, 5% ethanedithiol and 90% TFA for 16 h at ambient
temperature with stirring.

Purification

10  Crude peptide is purified by reverse phase
chromatography using either a C4 or C18 column and the
Buffer system:  Buffer A - 0.1% aqueous TFA, Buffer B -
80% Acetonitrile and 20% A.

N-Terminal Acetylation

15  The peptide resin obtained after the synthesis (with
Fmoc removed in the usual manner was) placed in a 0.3 MDMF
solution of 10 equivalents of Ac-OHSu for 60 minutes.  The
resin was filtered, washed with DMF, CH2C12, ether and
used in the next step.

20  Cyclization

The purified and lyophilized bis-S-(acetamidomethyl)
cysteine peptide (100-400 mg) was dissolved in 5 mls of
methanol containing 1 ml of acetic acid.  This was added
dropwise to a 1 litre methanol solution containing 1 g of

25  iodine.

After 2 h reaction, the excess iodine was removed by
addition of a dilute sodium thiosulfate solution until the
colour turns to a pale yellow, methanol was removed in
vacuo at room temperature and the concentrated solution

30  was finally completely decolourised with dropwise addition
of sodium thiosulfate and applied immediately onto a
preparatively reverse phase chromatography column.

Synthesis of Peptides using the Boc-Strategy

Syntheses of these peptides were carried out on the

35  ABI 430A instrument using polystyrene based resins.  For
peptide with C-terminal acids, the appropriate Merrified

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resin Boc-amino acid-O-resin or the 100-200 mesh PAM resin
is used (7, 8, 19-21, 26, 31). Peptides with C-terminal
amides are synthesized on MBHA resins (32, 33).

Couplings of Boc-amino acids (Table 2) were carried
5 out either using symmetrical anhydride method or a HOBt
ester method mediated by DCC or HTBU.

TABLE 2

| Amino Acid | Protecting Group | Coupling Method |
|---|---|---|
| Arg | Tos | HOBt or S.A. |
| Asp | Cxl, OBzl | HOBt or S.A. |
| Cys | 4-MeBzl | HOBt or S.A. |
| Glu | Cxl | HOBt or S.A. |
| His | Dnp, Bom | HOBt or S.A. |
| Lys | 2-ClZ | HOBt or S.A. |
| Ser | Bzl | HOBt or S.A. |
| Thr | Bzl | HOBt or S.A. |
| Tyr | Br-Z | HOBt or S.A. |
| Asn | Xan | HOBt or S.A. |
| Gln | none | HOBt only |

(line numbers 10, 15, 20 in margin)

Cleavage

Peptides were cleaved in HF with p-cresol or anisole
as scavenger for up to 90 min. For His with Dnp
protection, the resin required pre-treatment with
25 mercaptoethanol:DIPEA:DMF (2:1:7), for 30 min. After
removal of scavengers by ether wash, the crude peptide is
extracted into 30% acetonitrile in water.

N-Terminal Acetylation

Acetylation was achieved by treating the deblocked
30 resin with acetic anhydride in DMF solution.

TABLE 3

| No | hTNF | Sequence |
|---|---|---|
| 1 | 1-18 | VAL ARG SER SER SER ARG THR PRO SER ASP LYS PRO VAL ALA HIS VAL VAL ALA |
| 2 | 6-18 | ARG THR PRO SER ASP LYS PRO VAL ALA HIS VAL VAL ALA |

(line numbers 35 in margin)

|    | 3  | 2-15      | ART SER SER SER ARG THR PRO SER ASP LYS PRO VAL ALA HIS |
|----|----|-----------|---------------------------------------------------------|
|    | 4  | 1-26      | VAL ARG SER SER SER ARG THR PRO SER ASP LYS PRO VAL ALA HIS VAL VAL ALA ASN PRO GLN ALA GLU GLY GLN LEU |
| 5  |    |           |                                                         |
|    | 5  | 10-18     | ASP LYS PRO VAL ALA HIS VAL VAL ALA                     |
|    | 6  | 15-22     | HIS VAL VAL ALA ASN PRO GLN ALA                         |
|    | 7  | 6-16      | ARG THR PRO SER ASP LYS PRO VAL ALA HIS VAL             |
| 10 | 8  | 6-17      | ARG THR PRO SER ASP LYS PRO VAL ALA HIS VAL VAL         |
|    | 9  | 8-16      | PRO SER ASP LYS PRO VAL ALA HIS VAL                     |
|    | 10 | 8-15      | PRO SER ASP LYS PRO VAL ALA HIS                         |
|    | 11 | 8-15      | PRO SER ASP LYS PRO VAL ALA                             |
| 15 | 12 | 8-13      | PRO SER ASP LYS PRO VAL                                 |
|    | 13 | 7-18      | THR PRO SER ASP LYS PRO VAL ALA HIS VAL VAL ALA         |
|    | 14 | 8-18      | PRO SER ASP LYS PRO VAL ALA HIS VAL VAL ALA             |
| 20 | 15 | 9-18      | SER ASP LYS PRO VAL ALA HIS VAL VAL ALA                 |
|    | 16 | 11-18     | LYS PRO VAL ALA HIS VAL VAL ALA                         |
|    | 17 | 12-18     | PRO VAL ALA HIS VAL VAL ALA                             |
|    | 18 | 12-18     | Ac PRO VAL ALA HIS VAL VAL ALA NH2                      |
|    | 19 | 6-18      | ARG THR PRO SER ALA LYS PRO VAL ALA HIS VAL VAL ALA     |
| 25 |    | Ala(10)   |                                                         |
|    | 20 | 6-18      | ARG THR PRO SER ASP ALA PRO VAL ALA HIS VAL VAL ALA     |
|    |    | Ala(11)   |                                                         |
| 30 | 21 | 6-18      | ARG THR PRO SER LYS ASP PRO VAL ALA HIS VAL VAL ALA     |
|    |    | Lys(10)   |                                                         |
|    |    | Asp(11)   |                                                         |
|    | 22 | 1-18      | VAL ARG SER SER SER ARG THR PRO SER ASP LYS PRO VAL ALA ARG VAL VAL ALA |
| 35 |    |           |                                                         |
|    |    | Arg(15)   |                                                         |

|  |  |  |  |
|---|---|---|---|
| | 23 | 1-18 | VAL ARG SER SER SER ARG THR PRO SER ASP |
| ~ | | GLN(15) | LYS PRO VAL ALA GLN VAL VAL ALA |
| | 24 | 1-18 | VAL ARG SER SER SER ARG THR PRO SER ASP |
| | | Leu(14) | LYS PRO VAL LEU HIS VAL VAL ALA |
| 5 | 25 | 1-18 | VAL ARG SER SER SER ARG THR PRO SER ASP |
| | | | LYS PRO VAL VAL HIS VAL VAL ALA |
| | | Val(14) | |
| | 26 | 6-26 | ARG THR PRO SER ASP LYS PRO VAL ALA HIS |
| | | | VAL VAL ALA ASN PRO GLN ALA GLU GLY GLN |
| 10 | | | LEU |
| | 27 | 1-16 | VAL ARG SER SER SER ARG THR PRO SER ASP |
| | | | LYS PRO VAL ALA HIS VAL |
| | 28 | 1-10 | VAL ARG SER SER SER ARG THR PRO SER ASP |
| | 29 | 8-14 | Ac PRO SER ASP LYS PRO VAL ALA NH2 |
| 15 | 30 | 6-16 | Ac ARG THR PRO SER ASP LYS PRO VAL ALA |
| | | | HIS VAL NH2 |
| | 31 | 6-16 | ARG THR PRO SER ASP LYS PRO VAL VAL HIS |
| | | | VAL |
| | | Val(14) | |
| 20 | 32 | 6-16 | ARG THR PRO SER ASP LYS PRO VAL ALA HIS |
| | | | ALA |
| | | ALA(16) | |
| | 33 | 6-16 | ARG THR PRO SER ASP LYS PRO VAL ALA ALA |
| | | | VAL |
| 25 | | ALA(15) | |
| | 34 | 6-16 | ART THR PRO SER ASP LYS PRO VAL ALA LYS |
| | | | VAL |
| | | LYS(15) | |
| | 35 | 6-16 | ARG THR PRO SER ASP LYS PRO VAL ALA ASP |
| 30 | | | VAL |
| | | ASP(15) | |
| | 36 | 6-16 | ARG THR PRO SER ASP LYS PRO VAL ALA D-HIS |
| | | | VAL |
| | | D-HIS(15) | |
| 35 | 275 | 111-120 | ALA LYS PRO TRP TYR GLU PRO ILE TYR LEU |

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302  43-48      LEU ARG ASP ASN GLN LEU VAL VAL PRO SER
                SLU GLY LEU TYR LEU ILE

303  94-109     LEU SER ALA ILE LYS SER PRO LYS GLN ARG
                GLU THR PRO GLU GLY ALA

304  63-83      LEU PHE LYS GLY GLN GLY CYS PRO SER THR
                HIS VAL LEU LEU THR HIS THR ILE SER ARG
                ILE

305  132-150    LEU SER ALA GLU ILE ASN ARG PRO ASP TYR
                LEU ASP PHE ALA GLU SER GLY GLN VAL

306  13-26      VAL ALA HIS VAL VAL ALA ASN PRO GLN ALA
                GLU GLY GLN LEU

307  22-40      ALA GLU GLY GLN LEU GLN TRP LEU ASN ARG
                ARG ALA ASN ALA LEU LEU ALA ASN GLY

308  54-68      GLY LEU TYR LEU ILE TYR SER SLN VAL LEU
                PHE LYS GLY GLN GLY

309  73-94      HIS VAL LEU LEU THR HIS THR ILE SER ARG
                ILE ALA VAL SER TYR GLN THR LYS VAL ASN
                LEU LEU

323  79-89      THR ILE SER ARG ILE ALA VAL SER TYR GLN
                THR

347  132-157    LEU SER ALA GLU ILE ASN ARG PRO ASP TYR
                LEU ASP PHE ALA GLU SER GLY GLN VAL TYR
                PHE GLY ILE ILE ALA LEU
```

Endothelial Cell Clotting Assays

Endothelial cell procoagulant activity (PCA)
induction by TNFα was determined using bovine aortic
endothelial cells (BAE) according to the procedure of
Bevilacqua et al., 1986 PNAS 83, 4522 with the following
modifications:  BAE cells were propagated in McCoys 5A
medium supplemented with 10% FCS, penicillin, streptomycin
and L-gutamine in standard tissue culture flasks and
24-well dishes.  TNFα treatment of culture (3μg/ml) was
for 4 hours at 37°C in the presence of growth medium
after which the cells were washed and scrape-harvested
before being frozen, thawed and sonicated.  Total cellular
PCA was determined in a standard one-stage clotting assay

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using normal donor platelet poor plasma to which 100μl of
CaCl$_2$ and 100μl of cell lystate was added. Statistical
significance was determined by unpaired t-test.

Neutrophil Activation Studies

5          In these experiments, neutrophils were prepared from
blood of healthy volunteers by the rapid single step
method (Kowanko and Ferrante 1987 Immunol 62, 149). To
100μl of 5 x 10$^6$ neutrophils/ml was added 100μl of
either 0, 10, 100μg of peptide/ml and 800μl of

10    lucigenin (100μg). The tubes were immediately placed
into a light proof chamber (with a 37$^o$C water jacket
incubator) of a luminometer (model 1250; LKB Instruments,
Wallac, Turku, Finaldn). The resultant light output (in
millivolts was recorded). The results are recorded as the

15    maximal rate of chemiluminescence production.

Effects of peptides on neutrophil chemiluminescence
induced by either TNF or LPS: Neutrophils of 96-99%
purity and )99% viability were prepared from blood of
normal healthy volunteers by centrifugation (400g for 30

20    min) through Hypaque-Ficoll medium of density 1.114.
Following centrifugation the neutrophils formed a single
band above the erythrocytes and 1 cm below the mononuclear
leukocyte band. These were carefully recovered and washed
in medium 199. To assess the lucigenin-dependent

25    chemiluminescence response 100ul of 5 x 10$^6$
neutrophils/ml was added 100ul of either 0,1,10,100ug of
peptide/ml and TNF or LPS and 800ul of lucigenin (100ug).
The tubes were immediately placed into a light proof
chamber with a 37$^o$C water jacket incubator of a

30    luminometer. The resultant light output (in millivolts)
was recorded. The results are recorded as the maximal of
chemiluminescence production. In experiments which
examined the ability of the peptides to prime for the
response to fMLP, 100ul of 5 x 10$^5$ neutrophils /ml

35    preincubated in peptide and LPS or TNF for 20 mins was

added to 100ul of diluent or fMLP ($5 \times 10^{-6}$M) before the
addition of 700ul of lucigenin (100ug). The
chemiluminescence was measured as above. Neutrophils from
at least three individuals were used in triplicate
5  determinations of anti-TNF or LPS activity. Results were
deemed positive if at least 50% inhibition of
chemiluminescence was obtained in at least two thirds of
cases.

WEH1-164 Cytoxicity

10     Bioassay of recombinant TNF activity was performed
according to the method described by Espevik and
Nissen-Meyer. (Espevik and Nissen-Meyer 1986 J. Immunol.
Methods 95 99-105)

Tumour Regression Experiments

15     Subcutaneous tumours were induced by the injection of
approximately $5 \times 10^5$ WEH1-164 cells. This produced
tumours of diameters of 10 to 15mm approximately 14 days
later. Mice were injected i.p. with recombinant human TNF
(10μg and 20μg) and peptide (1mg) for four consecutive
20  days. Control groups received injections of PBS. Tumour
size was measured daily throughout the course of the
experiment. Statistical significance of the results was
determined by unpaired Student T-test.

Radioreceptor assays

25     WEH1-164 cells grown to confluency were scrape
harvested and washed once with 1% bovine serum albumin in
Hanks balanced salt solution (HBSS, Gibco) and used at
$2 \times 10^6$ cells pre assay sample. For the radioreceptor
assay, the cells were incubated with varying amounts of
30  either unlabelled TNFα($1-10^4$ ng per assay sample) or
peptide ($0-10^5$ ng per assay sample) and $^{125}$I-TNF
(50,000cpm) for 3 hours at $37^\circ$C in a shaking water
bath. At the completion of the incubation 1ml of HBSS/BSA
was added to the WEH1-164 cells, the cells spun and the
35  bound $^{125}$I in the cell pellet counted. Specific binding

was calculated from total binding minus non-specific binding of triplicate assay tubes. 100% specific binding corresponded to 1500 cpm.

In Vivo Studies of TNF Toxicity

5    Mice were administered with either TNF (200μg), Peptide 1 (10mg) and TNF (200μg)+Peptide 1 (10mg) via intravenous injection. Blood glucose levels and appearance of the animals was evaluated at 15, 30, 60, 120, 180 minutes after injection. Appearance parameters

10   which were evaluated included ruffling of fur, touch sensitivity, presence of eye exudate, light sensitivity and diarrhoea.

Infection of mice with malaria parasites and treatment with TNF+ Peptide 1

15   All the mice used were male, CBA/CaH stain and 6-8 weeks old. P. vinkei vinkei (Strain V52, from F.E.G. Cox, London) has undergone several serial passages in CBA mice, after storage in liquid nitrogen, before use in these experiments. Infections were initiated by intraperitoneal

20   injection of $10^6$ parasitized erythrocytes. Mice were treated with TNF(7μg) ± peptide (8.3 mg) administered iv.

Assays for blood glucose

Nonfasting blood glucose levels were determined on a Beckman Glucose Analyzer 2 (Beckman Instruments) or on a

25   Exectech blood glucose sensor (Clifford Hallam Pty. Ltd).

Reactive Nitrogen Intermediates (RNI)

RNI levels in blood were determined by the method of Rockett et al (1991) in-vivo induction of TNF, LT and IL-1 implies a role for nitric oxide in cytokine-induced

30   malarial cell-mediated immunity and pathology. J. Immunol. in press.

TNF and LPS Lethality Experiments: balb/C or balbC x swiss F1 mice carrying Meth A ascites tumours elicited by prior I.P. inoculation of 0.5μl pristane 7 days before

35   I.P. injection of tumour cells. Nine to ten days after

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inoculation with the tumour cells 25 ug of human
recombinant TNF was subcutaneously administered and a
short time later 1mg of either test peptide, bovine serum
albumen, phosphate buffered saline or neutralizing
5    anti-TNF MAb 47 was administered at a separate
subcutaneous site.  The number of surviving animals was
then observed at 18 hours and 24 hours post TNF
treatment.  In experiments which assessed the effects of
1-related peptides on on LPS lethality the mice were
10   administered 500ug E.coli LPS and peptide or other
treatment in a similar manner.  In LPS experiments
polymyxin B, an LPS inhibitor, replaced MAb 47 as a
positive control.  The number of animals surviving was
assessed at intervals up to 64 hours after LPS challenge.
15   Experiments in D-galactosamine sensitized mice:  Female
Bablb/C mice were co-injected intraperitoneally with 16 mg
D-galactosamine and 2ug human recombinant TNF.  The mice
were then injected subcutaneously with either test
peptide, phosphate buffered saline or neutralizing
20   anti-TNF monoclonal antibody 47.  The number of surviving
animals was assessed at intervals up to 48 hours after TNF
challenge.
RESULTS
          The results obtained with each of the peptides are
25   summarised in Table 4.  A single * indicates heightened
activity in that test whilst a double ** indicates
activity at low concentrations of peptide but not high
concentrations.

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## TABLE 4

| PEPTIDE | IN VIVO | | | DIRECT | IN VITRO NEUTROPHIL | | |
|---|---|---|---|---|---|---|---|
| | TNF TOXICITY METH A | TNF TOXICITY D-GAL | LPS TOXICITY METH A | | TNF PRIMING | DIRECT | LPS PRIMING |
| | + | + | + | + | + | + | + |
| 1 | + | + | + | + | | | |
| 2 | +* | + | + | +* | | | |
| 8 | - | | - | +** | | | |
| 9 | - | | - | + | | | |
| 10 | +* | - | - | - | | | |
| 11 | - | | | - | | | |
| 12 | + | | | - | | | |
| 16 | - | | | - | | | |
| 17 | - | | + | - | | | |
| 13 | - | | - | + | | | |
| 14 | - | | + | + | | | |
| 15 | - | | - | | | | |
| 18 | - | - | | - | + | + | + |
| 19 | + | | + | + | | | |
| 20 | - | | - | - | + | + | + |
| 21 | +* | | + | + | + | | |
| 22 | | + | + | + | | | |
| 23 | + | + | + | - | | | |
| 24 | - | | + | + | | | |
| 25 | +/- | | - | + | | | |
| 26 | - | | - | + | | | |
| 4 | - | | | + | | | |
| 5 | - | | - | - | | | |
| 6 | - | | | - | | | |
| 3 | - | | | | | | |
| 28 | + | - | + | | | | |
| 29 | - | + | + | | | | |
| 30 | +* | + | + | | | | |
| 31 | + | + | - | | | | |
| 32 | - | | +* | | | | |
| 33 | - | | +* | | | | |
| 34 | - | | - | | | | |
| 36 | - | | + | | | | |
| 35 | + | | - | | | | |
| 27 | - | | + | | | | |
| 7 | - | | + | +* | | | |

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TNF administered at a dose of 200µg was found to be
toxic in mice according to the parameters studied.  In
particular, blood glucose levels had fallen by 120 minutes
(Fig 7) Peptide 1 alone in 2 of the 3 mice studied did not
5    reduce blood glucose levels.  Mouse 1 in this group
recovered normal blood glucose levels within by
180 minutes.  Mice in the group treated with a combination
of TNF and Peptide 1 showed no reduction in blood glucose
levels at 120 min and a small decrease at 180 min.

10       As shown in Fig. 6, 10µg of Peptide 2 given to mice
treated with 200µg of recombinant human TNF abrogated TNF
toxicity as indicated by the inhibition of blood glucose
changes evident in mice treated with TNF alone.

When general appearance of treated mice was
15   considered it was noted that all 3 TNF only treated mice
had ruffled fur, touch sensitivity and light sensitivity.
One mouse in this group also had diarrhoea.  Mice treated
with Peptide 1 alone showed only slight touch sensitivity
with one mouse showing slight ruffling of the fur at 180
20   mins.  Mice treated with a combination of TNF and Peptide
1 showed ruffling of the fur and slight touch sensitivity
at 180 mins but failed to show either light sensitivity or
onset of diarrhoea.  In addition, Peptide 1 and related
peptides prevented death in acute models of TNF tethality
25   (Figs. 12 & 13).

Peptide 1 failed to either activate the respiratory
burst of human neutrophils (Table 5) or to induce
procoagulant activity on bovine aortic endothelial cells,
and hence is free of these negative aspects of TNF
30   activity in acute or chronic inflammation.  However,
Peptide 1 and related peptides inhibited both the TNF and
LPS-induced respiratory burst of human neutrophils (Figs.
15, 19, 18, 21).  Further, several peptides inhibited
priming of the neutrophil response to a
35   bacterially-derived peptide EMLP (Figs. 16, 17, 20, 22).

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### TABLE 5

| Peptide | Concentration $\mu g/10^6$ cells) | | | | |
|---------|------|------|------|------|------|
|         | 0    | 1    | 10   | 100  | 500  |
| 275     | 1.02 | 0.99 | 0.69 | 0.43 | 0.80 |
| 1       | 0.34 | 0.93 | 0.74 | 0.55 | 1.10 |
| 302     | 0.37 | 0.15 | 0.18 | 0.29 |      |
| 303     | 0.37 | 0.22 | 0.17 | 0.22 |      |
| 304     | 0.37 | 0.18 | 0.43 | 2.56 | 2.76 |
| 305     | 0.37 | 0.27 | 0.36 | 0.24 |      |
| 306     | 0.37 | 0.27 | 0.35 | 0.23 |      |
| 307     | 0.37 | 0.35 | 0.37 | 0.42 |      |
| 323     | 0.37 | 0.23 | 0.17 | 0.47 |      |
| 308     | 0.37 | 0.91 | 1.80 | 49.52|      |
| 309     | 0.37 | 0.38 | 0.98 | 13.44|      |

Results are expressed as mV of lucigenin dependent
chemiluminescence and represent peak of response i.e. the
maximal cell activity attained.

The results shown in Fig. 3 clearly show one of the
desirable effects of TNFα, i.e. tumour regression, is
unaffected by Peptide 1. Further, Peptide 1 does not
inhibit binding of TNF to tumour cell receptors (Fig 4).
Table 6 indicates that Peptide 1 is devoid of intrinsic
anti-tumour activity. The ability of Peptide 1 to prevent
high plasma RNI levels in TNFα treated malaria primed
mice is also strongly indicative of the therapeutic
usefulness of this peptide (Fig 5). Peptide 1 also
inhibits the TNF-induced decrease in blood glucose levels
evident in mice treated with TNF alone (Fig 2). Further
in the experiments involving mice infected with malaria
parasites; of the three mice treated with TNFα alone one
died and the other two were moribund. In contrast in the

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group of three mice treated with TNFα and Peptide 1 all
survived and none were moribund. This very marked result
also strongly indicates the potential usefulness of this
peptide as a therapeutic.

5      Peptide 1 inhibits not only the TNF-induced
hypoglycaemia in sensitized mice but also in ascites
tumour-bearing mice (Fig 8). Further, tumour-bearing mice
treated with TNF + Peptide 1 fail to develop the cachexia
or weight loss associated with TNF treatment (Fig 9).

10      As will be seen from the above information the
peptide of the present invention are capable of abrogating
TNF and/or LPS toxicity in vivo and neutrophil activation
by LPS or TNF in vitro. This peptide has utility in the
treatment of numerous disease states which are due to the

15 deleterious effects of TNF and/or LPS.

## TABLE 6

In vitro cytotoxicity of TNF and synthetic TNF peptides on
WEHI 164 fibrosarcoma cells

| TNF/PEPTIDE | % VIABLE CELLS* |
|---|---|
| TNF# | 26.6 |
| 275+ | 100 |
| 1 | 100 |
| 302 | 48.7 |
| 304 | 100 |
| 305 | 72.7 |
| 306 | 100 |
| 307 | 100 |
| 308 | 42.2 |
| 309 | 92.8 |

* %Viability was determined by comparison with untreated
control cells. Results shown are the means of
quadruplicate determinations.

35 # TNF was at 50 units per culture which is equivalent to
3ug (12ug/ml)

+ Each peptide was tested at 50ug/culture (200ug/ml)

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It will be appreciated by persons skilled in the art
that numerous variations and/or modifications may be made
to the invention as shown in the specific embodiments
without departing from the spirit or scope of the
5  invention as broadly described.  The present embodiments
are, therefore, to be considered in all respects as
illustrative and not restrictive.

CLAIMS:-
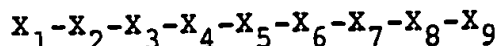
1. A linear or cyclic peptide of the general formula:-
   $X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9$
   in which

5      $X_1$ is null, Cys or $R_1$
       $X_2$ is null, Cys, $R_1$ or $A_1-A_2-A_3-A_4-A_5$
       in which    $A_1$ is Val or Ile or Leu or Met or His
                   $A_2$ is Arg or Cys or His
                   $A_3$ is Ser or Thr or Ala
10                 $A_4$ is Ser or Thr or Ala
                   $A_5$ is Ser or Thr or Ala
       $X_3$ is Cys, $R_1$ or $A_6-A_7$
       in which    $A_6$ is Arg or Cys or His or Absent
                   $A_7$ is Thr or Ser or Ala
15     $X_4$ is Cys, $R_1$ or $A_8-A_9$
       in which    $A_8$ is Pro or an Nα-alkylamino acid
                   $A_9$ is Ser or Thr or Ala
       $X_5$ is Cys, $R_1$ or $A_{10}$
       in which    $A_{10}$ is Asp or Ala or Cys or Glu or Gly
20  or Arg or His
       $X_6$ is Cys, $R_2$ or $A_{11}-A_{12}-A_{13}$
       in which    $A_{11}$ is absent or Cys or Arg or His or
                   Asp or Glu
                   $A_{12}$ is Pro or an Nα-alkylamino acid
25                 $A_{13}$ is Val or Ile or Phe or Tyr or Trp
                   or His or Leu or His or Met
       $X_7$ is null, Cys, $R_2$ or $A_{14}-A_{15}$
       in which    $A_{14}$ is Ala or Val or Gly or Ile or Phe
                   or Trp or Tyr or Leu or His or Met
30                 $A_{15}$ is absent or His or Arg or Glu or
                   Asn or Ala or Lys or Asp or Phe or Tyr or
                   Tap or Glu or Gln or Ser or Thr or Gly
       $X_8$ is null, Cys, $R_2$, $A_{16}$, $A_{16}-A_{17}$, $A_{16}-A_{17}-A_{18}$ or
       $A_{16}-A_{17}-A_{18}-A_{19}-A_{20}-A_{21}-A_{22}-A_{23}-A_{24}-A_{25}-A_{26}$

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in which $A_{16}$ is Val or Ile or Leu or Met or His

$A_{17}$ is Val or Ile or Leu or Met or His

$A_{18}$ is Ala or Gly

$A_{19}$ is Asp or Glu

$A_{20}$ is Pro or an Nα-alkylamino acid

$A_{21}$ is Gln or Asn

$A_{22}$ is Ala or Gly

$A_{23}$ is Glu or Asp

$A_{24}$ is Gly or Aln

$A_{25}$ is Gln or Asn

$A_{26}$ is Leu or Ile or Val or Met or His

$X_9$ is null, Cys or $R_2$

$R_1$ is R-CO, where R is H, straight, branched or cyclic alkyl up to C20, optionally containing double bonds and/or substituted with halogen, nitro, amino, hydroxy, sulfo, phospho or carboxyl groups (which may be substituted themselves), or aralkyl or aryl optionally substituted as listed for the alkyl and further including alkyl, or $R_1$ is glycosyl, nucleosyl, lipoyl or $R_1$ is an L- or D-α amino acid or oligomers thereof consisting of up to 5 residues

$R_1$ is absent when the amino acid adjacent is an unsubstituted desamino-derivative.

$R_2$ is

$-NR_{12}R_{13}$, wherein $R_{12}$ and $R_{13}$ are independently H, straight, branched or cyclic alkyl, aralkyl or aryl optionally substituted as defined for $R_1$ or N-glycosyl or N-lipoyl

$-OR_{14}$, where $R_{14}$ is H, straight, branched or cyclic alkyl, aralkyl or aryl, optionally substituted as defined for $R_1$

-O-glycosyl, -O-lipoyl or

- an L- or D-α-amino acid or a oligamu thereof consisting of up to 5 residues

or $R_2$ is absent, when the adjacent amino acid is a decarboxy derivative of cysteine or a homologue thereof or the peptide in a N-C cyclic form.

with the proviso that:

when $X_6$ is Cys or $R_2$ then $X_5$ is $A_{10}$, $X_4$ is $A_8$-$A_9$,
$X_3$ is $A_6$-$A_7$ and $X_2$ is $A_1$-$A_2$-$A_3$-$A_4$-$A_5$

when $X_5$ is Cys or $R_1$ then $X_6$ is $A_{11}$-$A_{12}$-$A_{13}$, $X_7$ is
5    $A_{14}$-$A_{15}$, $X_8$ is $A_{16}$-$A_{17}$-$A_{18}$ and $A_{11}$ is absent

when $X_4$ is Cys or $R_1$ then $X_5$ is $A_{10}$, $X_6$ is
$A_{11}$-$A_{12}$-$A_{13}$, $X_7$ is $A_{14}$-$A_{15}$ and $X_8$ is
$A_{16}$-$A_{17}$-$A_{18}$

when $X_2$ is $A_1$-$A_2$-$A_3$-$A_4$-$A_5$ then $X_8$ is not $A_{16}$

10  when $X_1$ is null, $X_2$ is Cys or $R_1$, $X_3$ is $A_6$-$A_7$, $X_4$ is
$A_8$-$A_9$, $X_5$ is $A_{10}$, $X_6$ is $A_{11}$-$A_{12}$-$A_{13}$, $X_7$ is
$A_{14}$-$A_{15}$ and $X_8$ is $A_{16}$ then $A_{16}$ is not D-His.

$X_1$ is always and only null when $X_2$ is $R_1$, Lys or Null

$X_2$ is always and only null when $X_3$ is Cys or $R_1$

15  $X_3$ is always and only null when $X_6$ is Cys or $R_2$

$X_7$ is always and only null when $X_7$ is Cys, $R_2$ or Null

$X_8$ is always and only null when $X_8$ is Cys, $R_2$ or Null

$X_9$ is always and only null when $X_8$ is Cys, $R_2$ or Null

when $X_1$ and $R_2$ are null, $X_3$ is $R_1$, $X_4$ is
20  $A_8$-$A_9$, $X_5$ is $A_{10}$, $X_6$ is $A_{11}$-$A_{12}$-$A_{13}$, $X_7$
is $A_{14}$-$A_{15}$, $X_8$ is $R_2$ and $A_{14}$ is Ala and $A_{15}$ is
absent then $R_1$ is acetyl and $R_2$ is $NH_2$.

2.    A linear or cyclic peptide as claimed in claim 1 in
which:-

25       $X_1$ is H, $X_2$ is $A_1$-$A_2$-$A_3$-$A_4$-$A_5$, $X_3$ is
$A_6$-$A_7$, $X_4$ is $A_8$-$A_9$, $X_5$ is $A_{10}$, $X_6$ is
$A_{11}$-$A_{12}$-$A_{13}$, $X_7$ is $A_{14}$-$A_{15}$, $X_8$ is
$A_{16}$-$A_{17}$-$A_{18}$ and $X_9$ is OH.

3.    A linear or cyclic peptide as claimed in claim 1 in
30  which:-

       $X_1$ is null, $X_2$ is H or Ac, $X_3$ is $A_6$-$A_7$,
$X_4$ is $A_8$-$A_9$, $X_5$ is $A_{10}$, $X_6$ is
$A_{11}$-$A_{12}$-$A_{13}$, $X_7$ is $A_{14}$-$A_{15}$, $X_8$ is
$A_{16}$-$A_{17}$-$A_{18}$ and $X_9$ is OH or $NH_2$.

4.    A linear or cyclic peptide as claimed in claim 1 in
which:-

$X_1$ is H, $X_2$ is $A_1$-$A_2$-$A_3$-$A_4$-$A_5$, $X_3$ is
$A_6$-$A_7$, $X_4$ is $A_8$-$A_9$, $X_5$ is $A_{10}$, $X_6$ is
OH and $X_6$, $X_7$ and $X_8$ are null.

5.    A linear or cyclic peptide as claimed in claim 1 in
which the peptide is selected from the group consisting
of:-

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-
Ala-His-Val-Val-Ala;

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val-Val-Ala;

Arg-Thr-Pro-Ser-Ala-Lys-Pro-Val-Ala-His-Val-Val-Ala;

Arg-Thr-Pro-Ser-Lys-Asp-Pro-Val-Ala-His-Val-Val-Ala;

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-
Ala-Arg-Val-Val-Ala;

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala
-Gln-Val-Val-Ala;

Ac-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val-NH2;

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-Ala-Val;

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-Lys-Val;

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val-Val;

Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val;

Pro-Ser-Asp-Lys-Pro-Val-Ala-His;

Pro-Ser-Asp-Lys-Pro-Val;

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Val
-His-Val-Val-Ala;

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val-Val-Ala-Asn
-Pro-Gln-Ala-Glu-Gly-Gln-Leu;

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp;

Ac-Pro-Ser-Asp-Lys-Pro-Val-Ala-NH2;

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-Asp-Val;

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-
Ala-His-Val-Val-Ala-Asn-Pro-Gln-Ala-Glu-Gly-Gln-Leu;

Asp-Lys-Pro-Val-Ala-His-Val-Val-Ala;

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val;

Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val-Val-Ala;

Pro-Sir-Asp-Lys-Pro-Val-Ala-His-Val-Val-Ala;

Pro-Val-Ala-His-Val-Val-Ala;  and

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Val-His-Val.

6.    A peptide as claimed in claim 5 in which the peptide is

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-
Ala-His-Val-Val-Ala;

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-His-Val-Val-Ala;

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp;

Arg-Thr-Pro-Ser-Ala-Lys-Pro-Val-Ala-His-Fal-Val-Ala;

Arg-Thr-Pro-Ser-Lys-Asp-Pro-Val-Ala-His-Val-Val-Ala;

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-
Ala-Arg-Val-Val-Ala;

Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-
Ala-Gln-Val-Val-Ala;  or

Arg-Thr-Pro-Ser-Asp-Lys-Pro-Val-Ala-Asp-Val.

7.    A pharmaceutical composition for use in treating subjects suffering from acute or chronic inflammation, the composition comprising a therapeutically effective amount of a peptide as claimed in any one of claims 1 to 6 and a pharmaceutically acceptable sterile carrier.

8.    A composition as claimed in claim 7 in which the composition is for administration topically, as a nasal spray, ocularly, intraveneously, intraperitoneally, intramuscularly, subcutaneously or for oral delivery.

9.    A composition as claimed in claims 7 or 8 in which the composition provides slow release of the active peptide.

10.  A method of treating a subject suffering from acute or chronic inflammation, the method comprising administering to the subject the composition as claimed in any one of claims 7 to 9.

11.  A method as claimed in claim 10 in which the subject is suffering from toxic shock, adult respiratory distress

syndrome, hypersensitivity pneumonitis, systemic lupus
erythromatosis, cystic fibrosis, asthma, bronchitis, drug
withdrawal, schistosomiasis, sepsis, rheumatoid arthritis,
acquired immuno-deficiency syndrome, multiple sclerosis,

5   leperosy, malaria, systemic vasculitis, bacterial
meningitis, cachexia, dermatitis, psoriasis, diabetes,
neuropathy associated with infection or autoimmune
disease, ischemia/reperfusion injury, encephalitis,
Guillame Barre Syndrome, atherosclerosis, chronic fatigue

10  syndrome, TB, other viral and parasitic diseases and OKT3
therapy.

12.   A method of ameliorating or reducing the adverse side
effects in a subject receiving cytotoxic drugs, cytokines,
immunopotentiating agents, radiation therapy and/or

15  chemotherapy comprising administering to the subject the
composition as claimed in any one of claims 7 to 9.

13.   An anti-idiotypic antibody to the peptide as claimed
in any one of claims 1 to 6, the anti-idiotypic antibody
being characterised in that it is capable of abrogating

20  TNF and/or LPS toxicity.

14.   A compound the three dimensional structure of which
is similar as a pharmacophore to the three dimensional
structure of the peptide as claimed in any one of claims 1
to 6, the compound being characterised in that it binds to

25  one or more antibodies raised against the peptides as
claimed in any one of claims 1 to 6 and that the compound
is capable of abrogating TNF and/or LPS toxicity.

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FIG. 1

VRSSSRTPSD10KPVAHVVANP20QAEGQLQWLN30RRA

NALLANG40VELRDNQLVV50PSEGLYLIYS60QVLFKGQGCP70STHVLL

THTI80SRIAVSYQTK90VNLLSAIKSP100CQRETREGAE110AKPWYEPI

YL120GGVFQLEKGD130RLSAEINRPD140YLDFAESGQV150YFGIIAL157

2/22



Fig 2

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Fig 3

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FIG. 4

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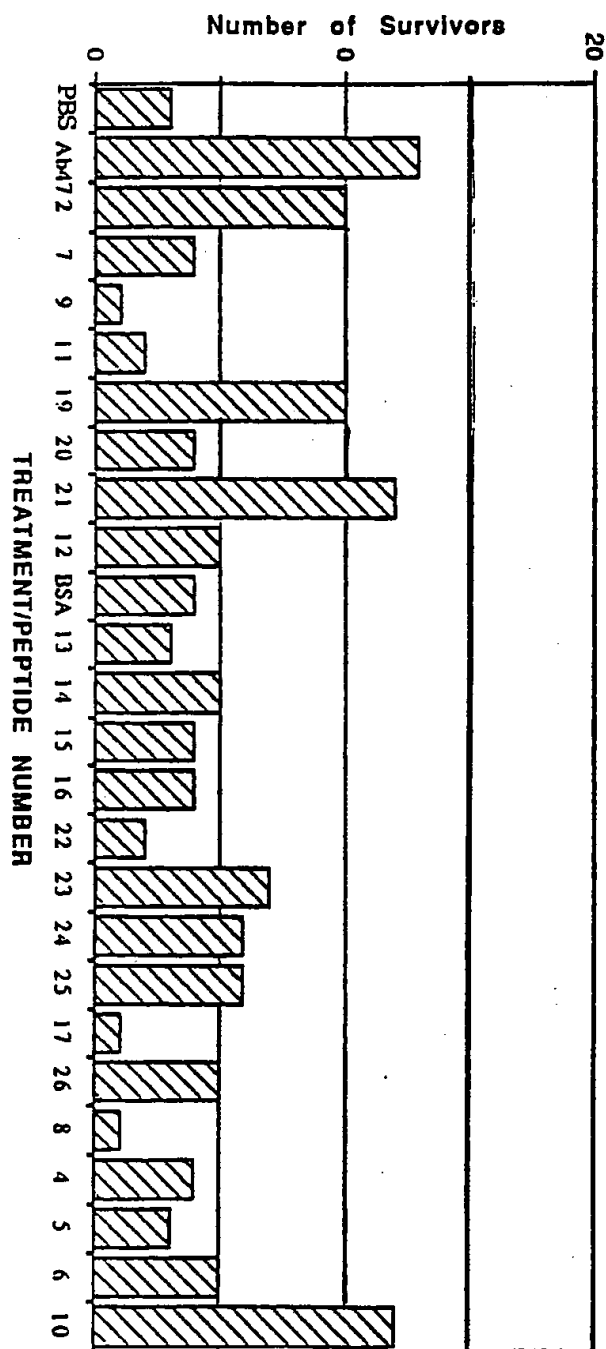

Fig 5

6/22
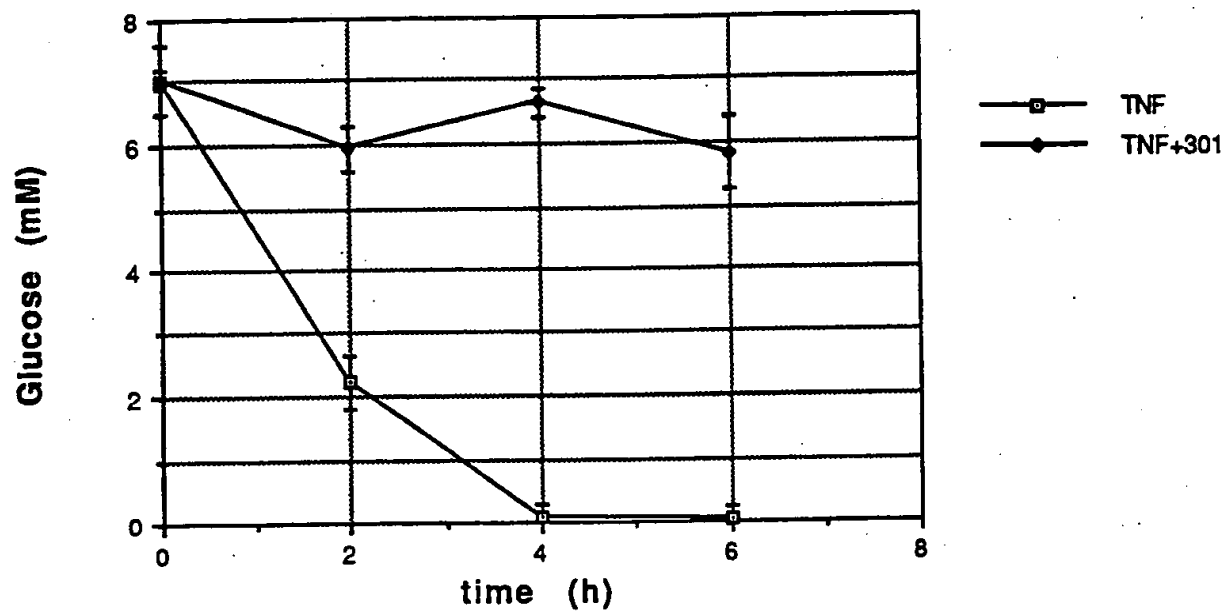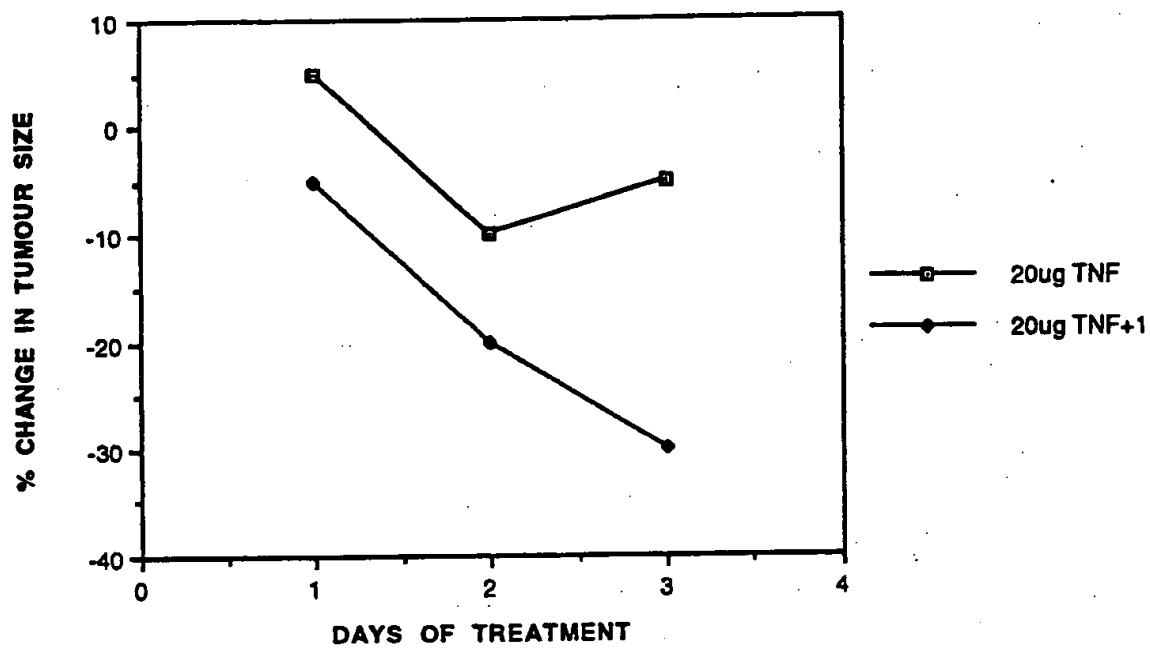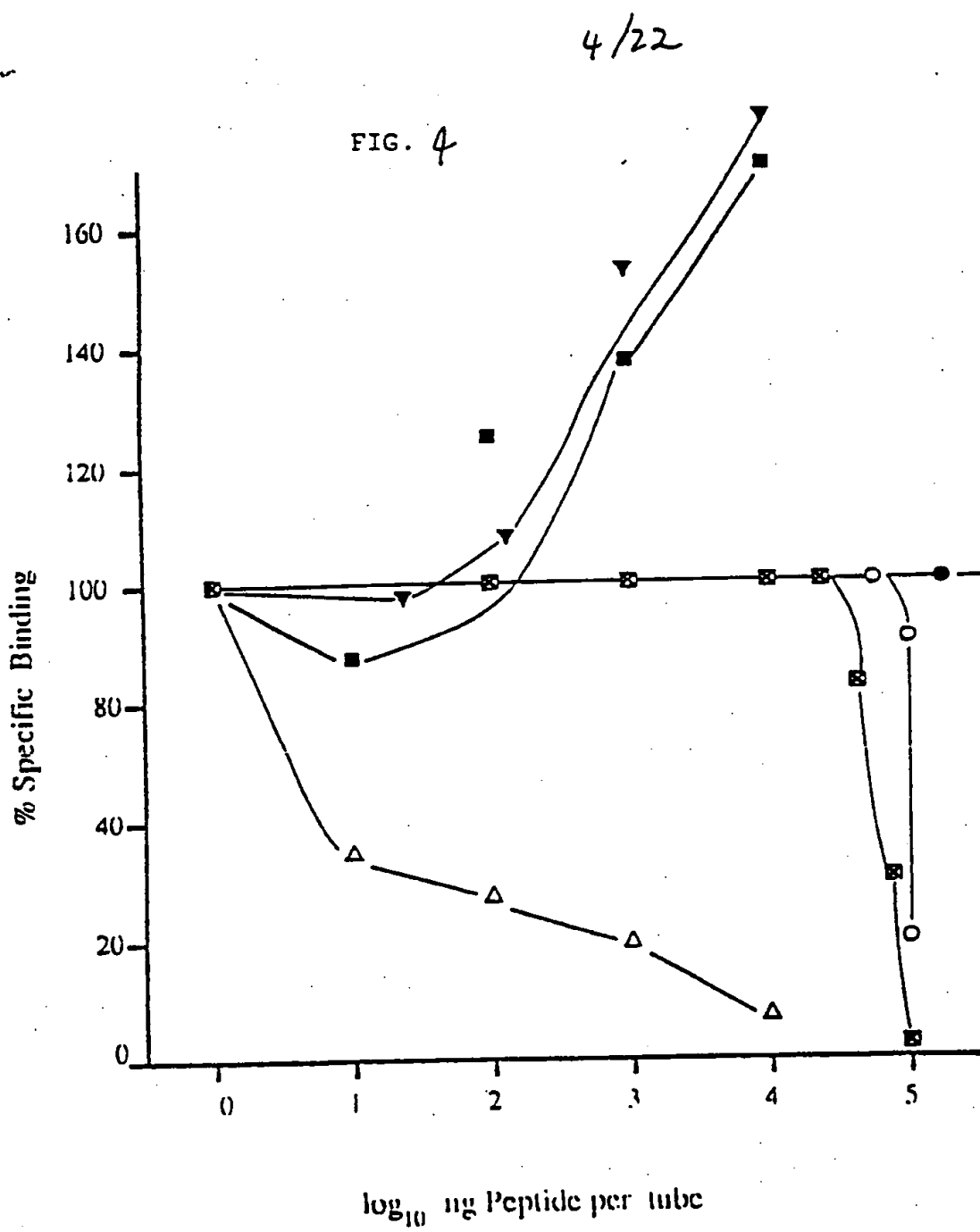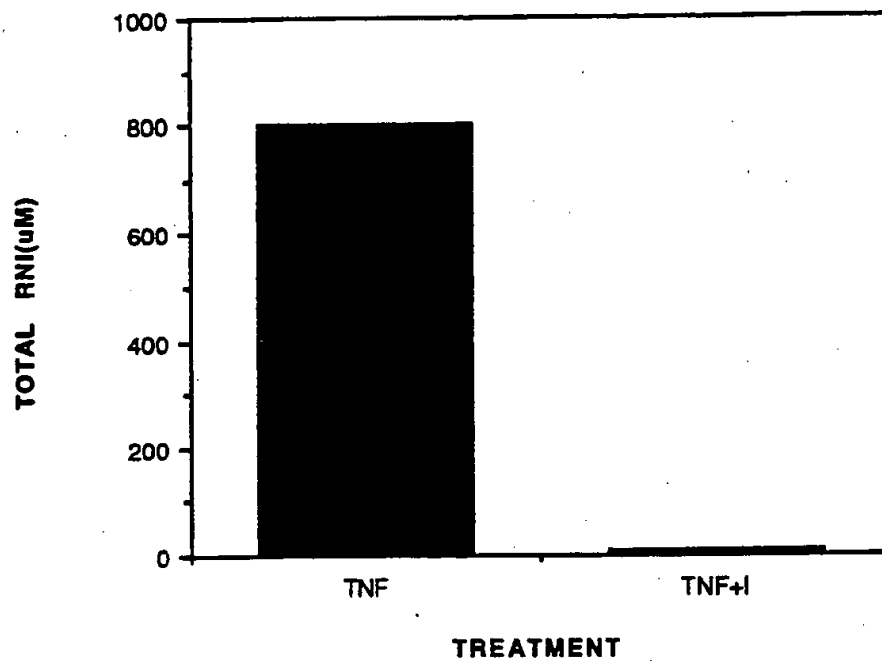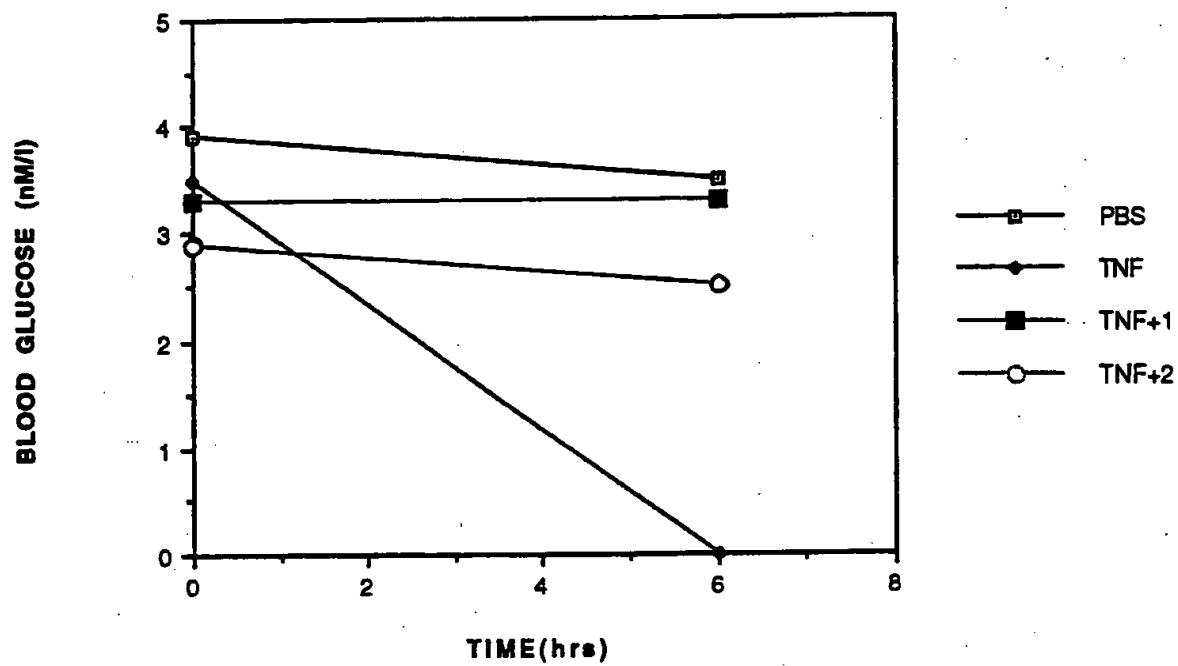


Fig 6
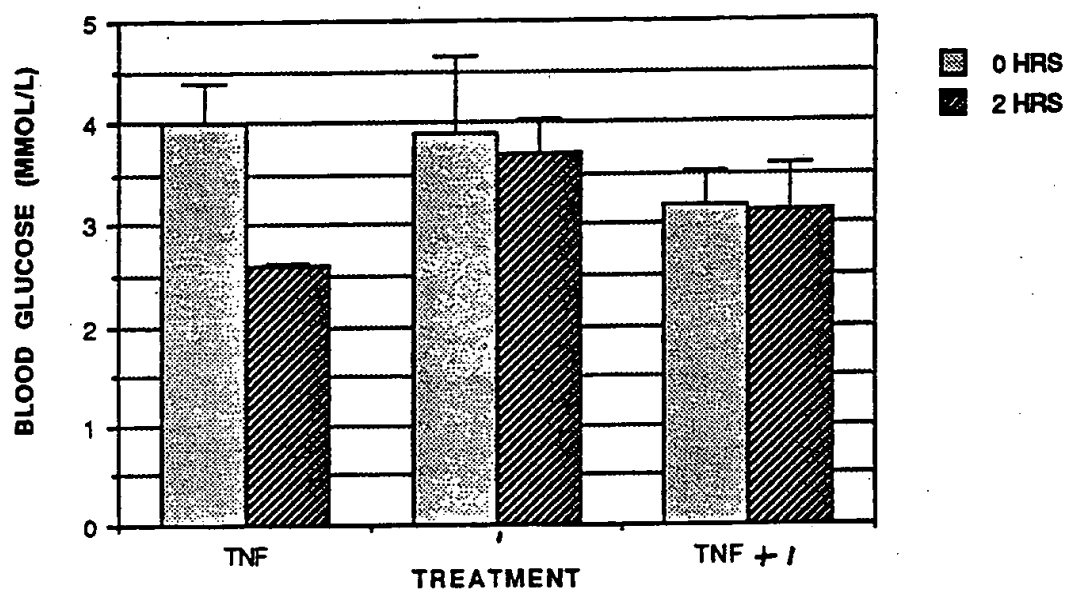
Fig 7
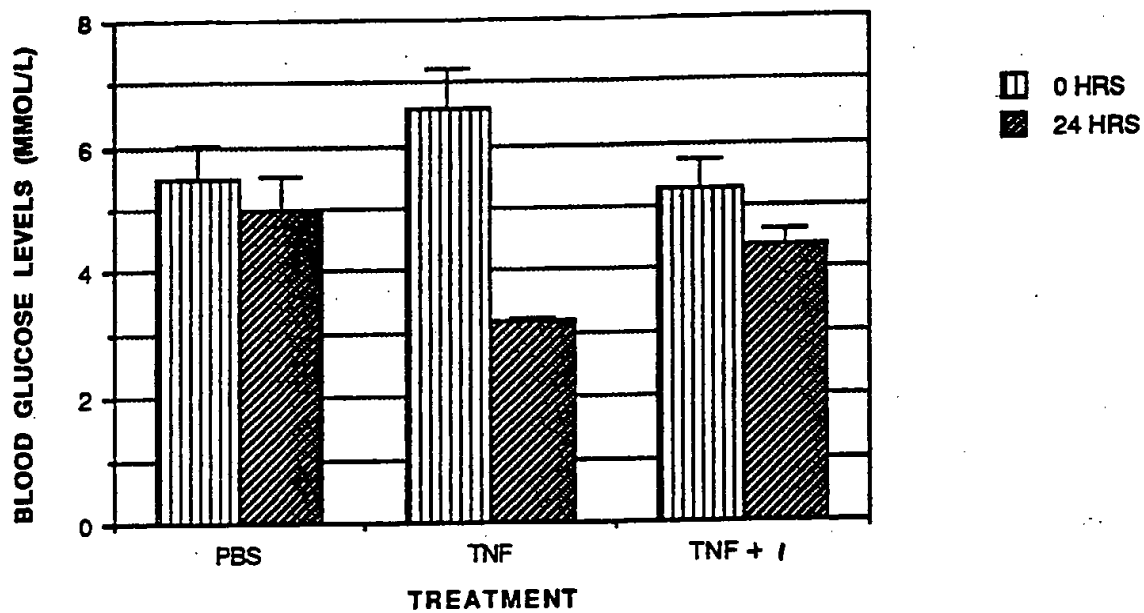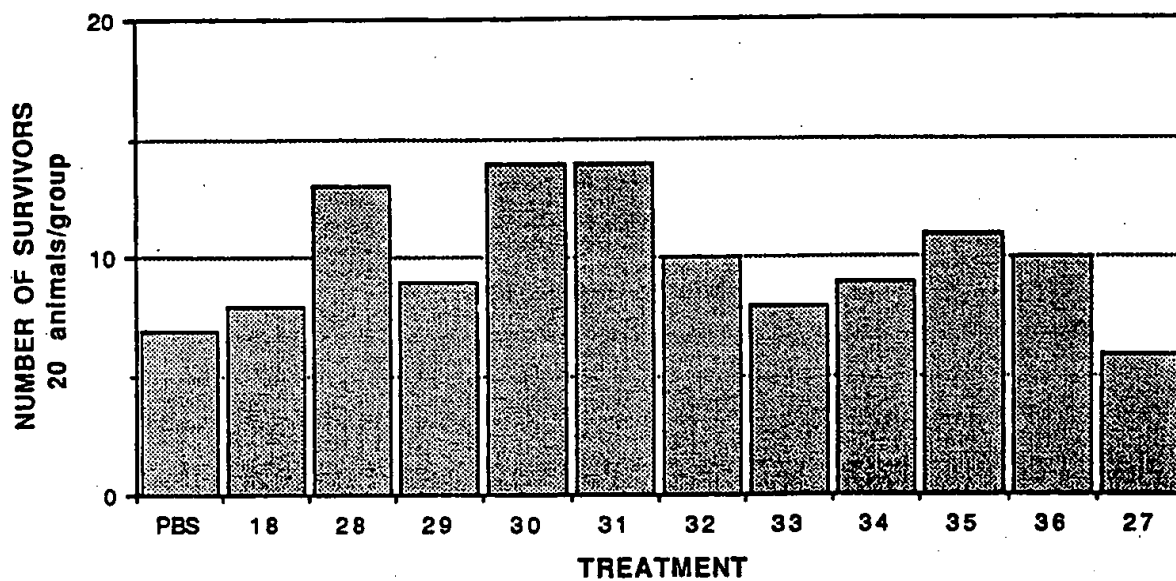
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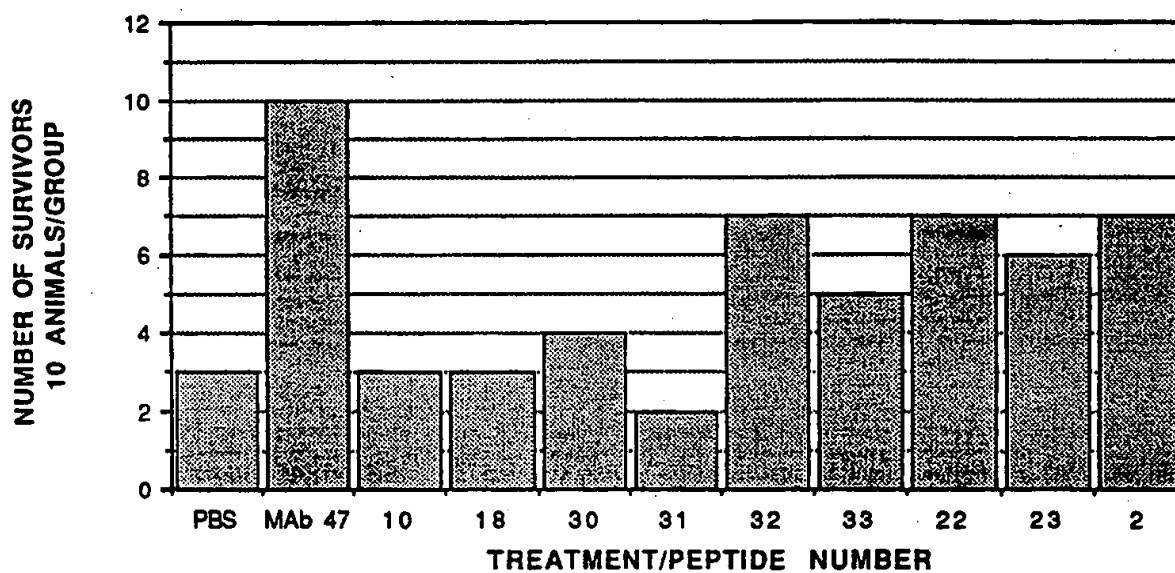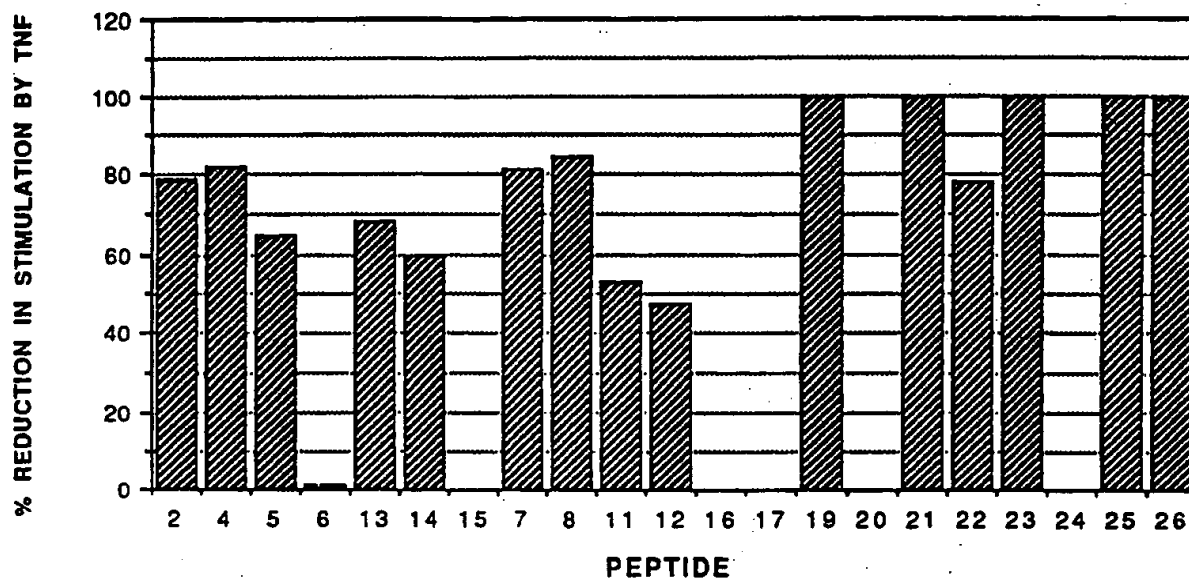
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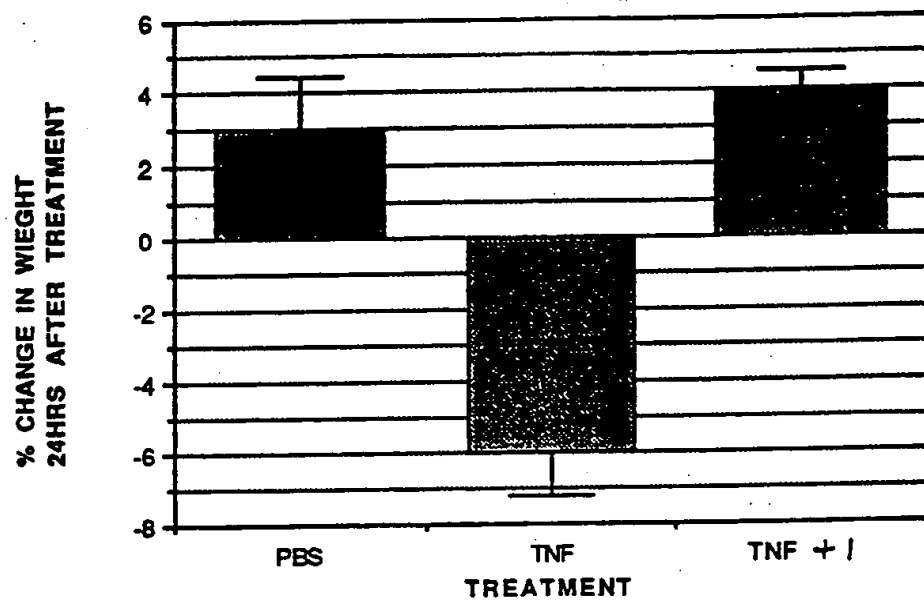


Fig 9

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Fig 10
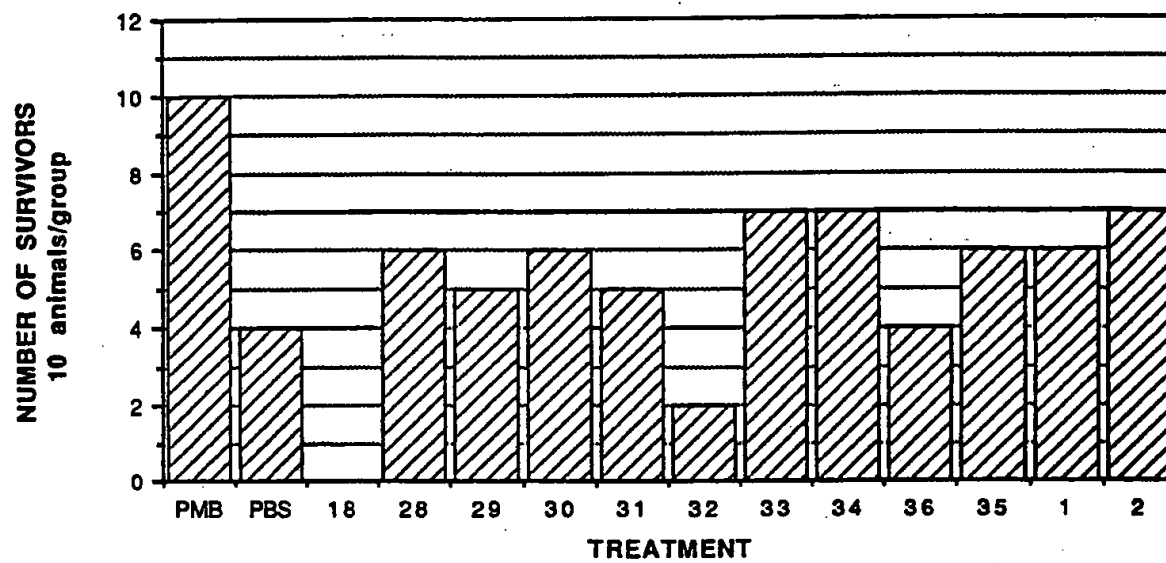
Fig 11
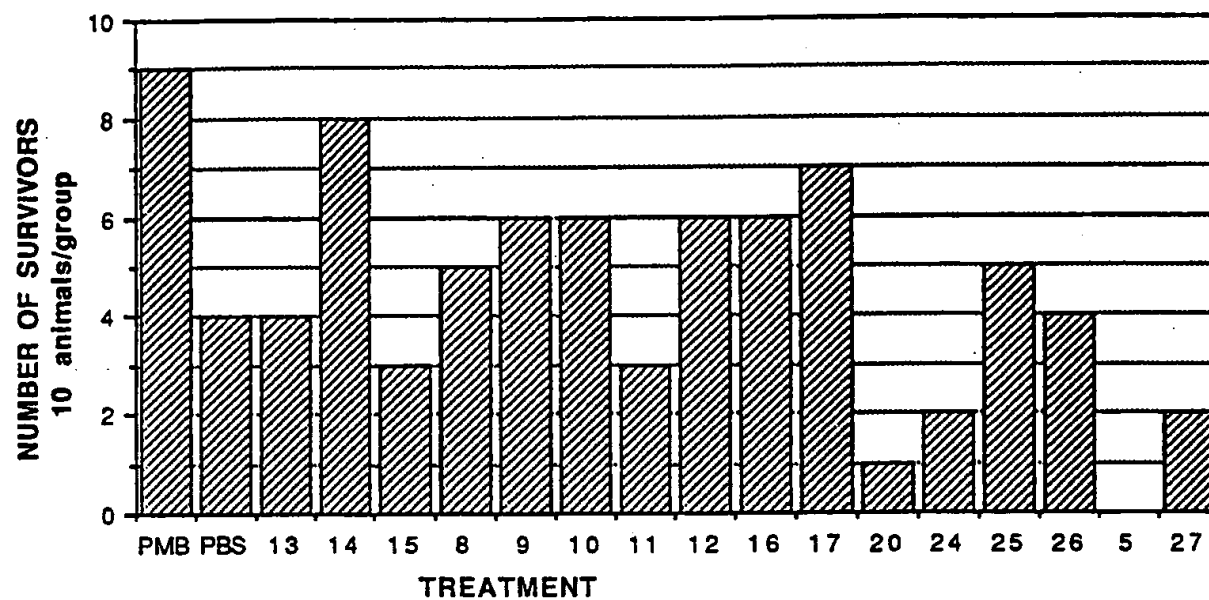
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FIG 12

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Fig 13
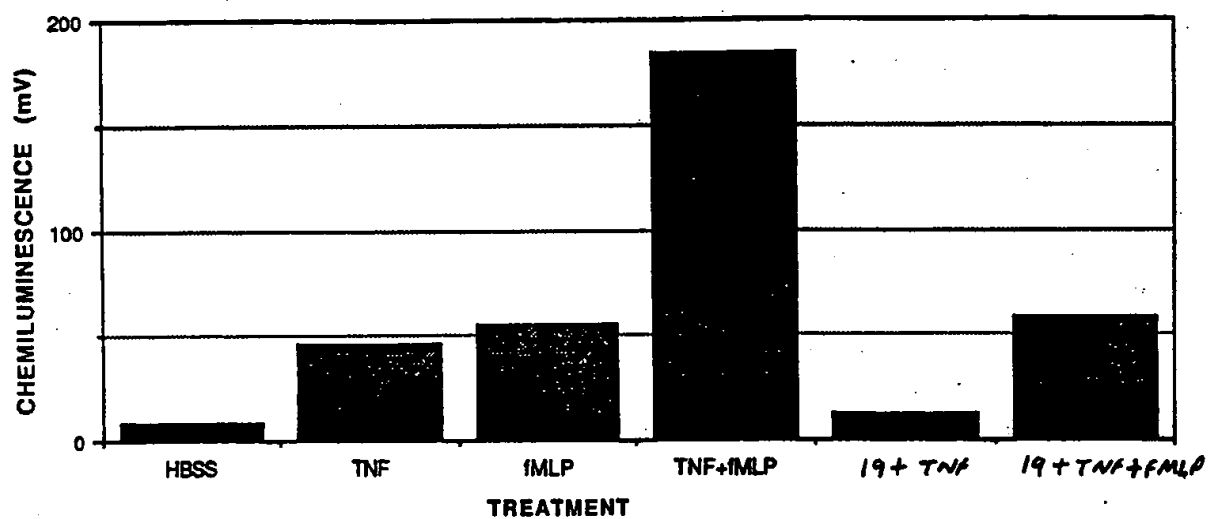
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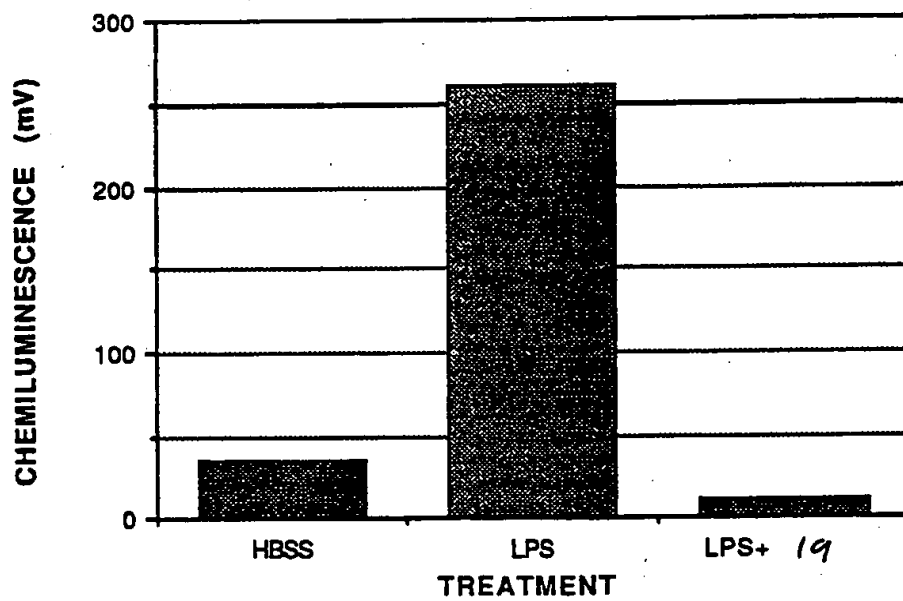
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F$_{IG}$ 15

FIG 16    16/22

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FIG 17

FIG 18

FIG. 19



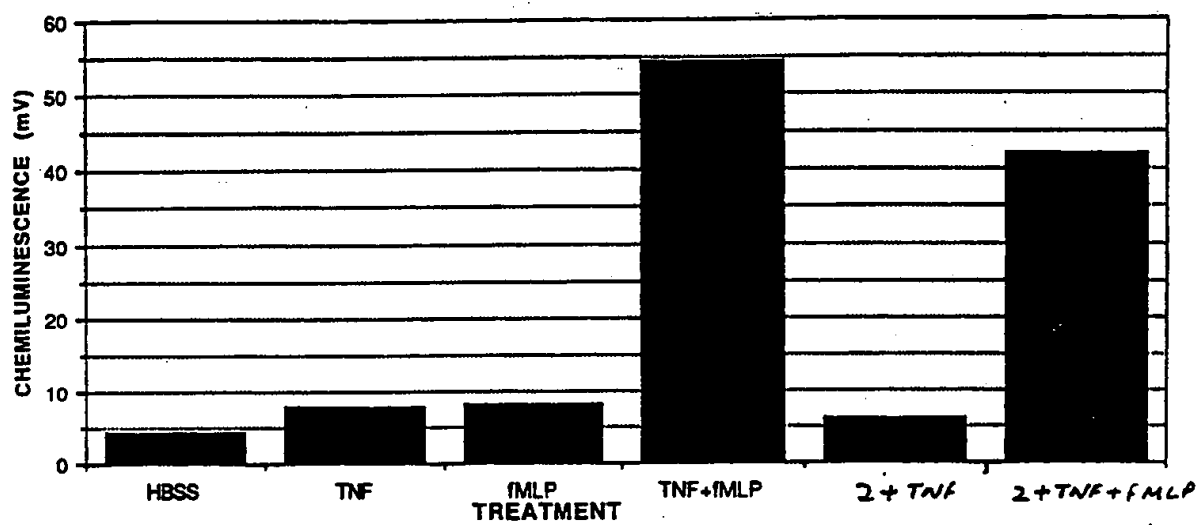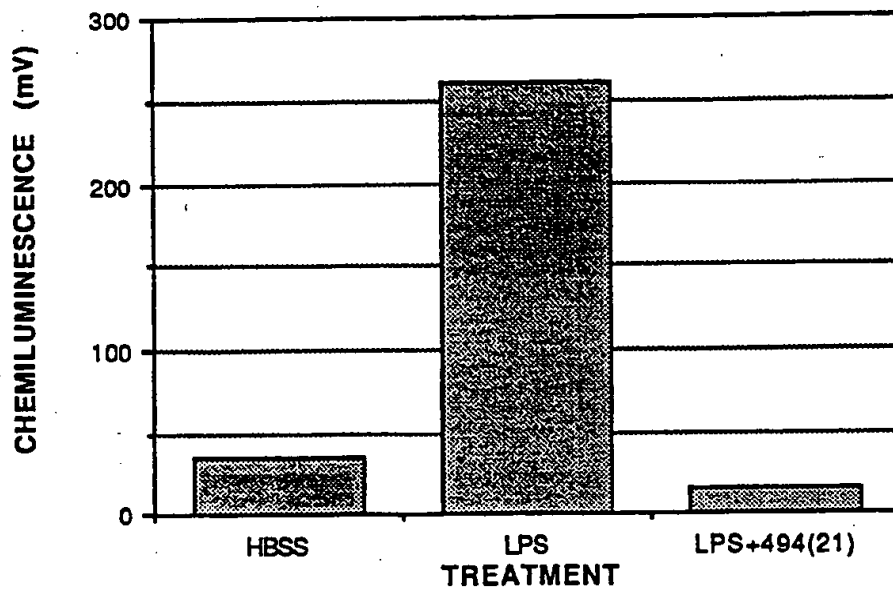CONCENTRATION OF PEPTIDE USED TO INHIBIT
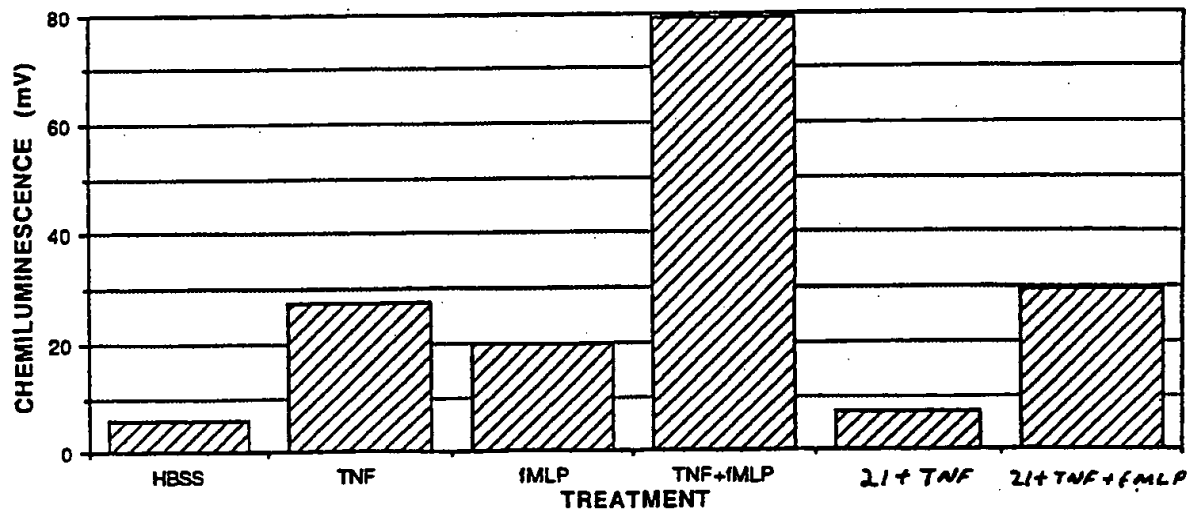TNF-INDUCED CHEMILUMINESCENCE
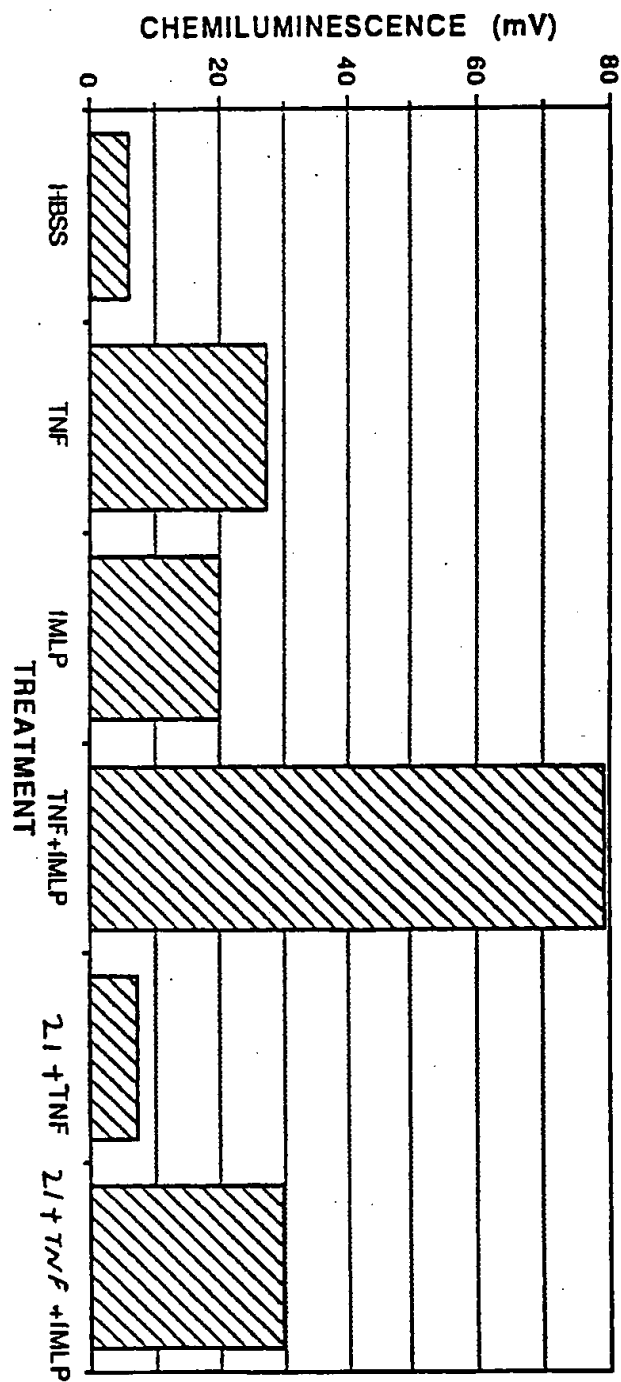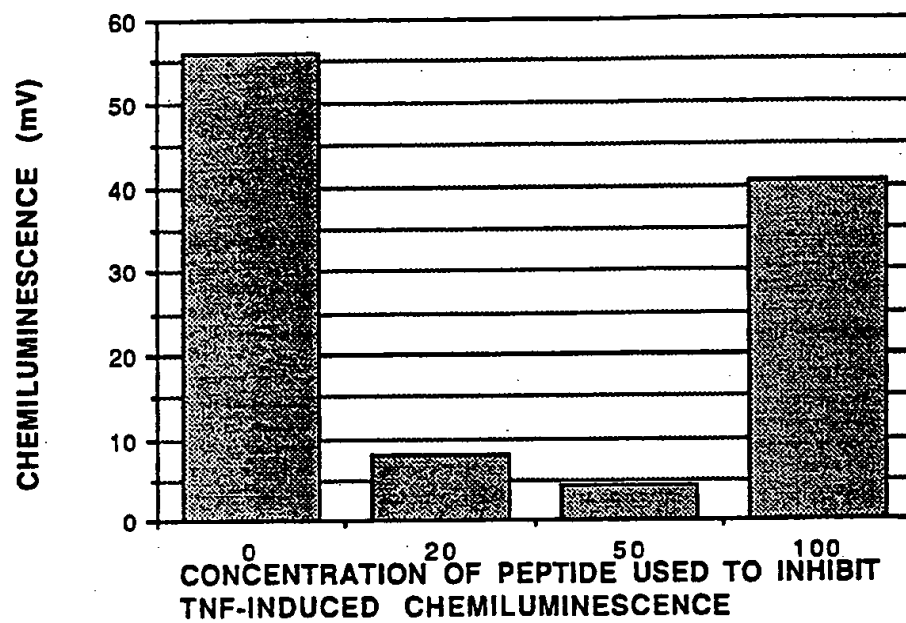
FIG 20

Fig 21

FIG. 22

# INTERNATIONAL SEARCH REPORT

## I.    CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)

According to International Patent classification (IPC) or to both National Classification and IPC

Int. Cl.⁵ C07K 7/06, 7/08, 7/10, A61K 37/02

## II.    FIELDS SEARCHED

### Minimum Documentation Searched [7]

| Classification System | Classification Symbols |
|---|---|
| IPC<br>Chem. Abs. online<br>CAS online registry | C07K 7/06, 7/08, 7/10, C07C 103/52.<br>Keywords: Tumo(u)r Necrosis Factor OR TNF<br>PROTEIN SEQUENCE SEARCH |

### Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

AU: IPC As Above

## III.    DOCUMENTS CONSIDERED TO BE RELEVANT [9]

| Category* | Citation of Document,[11] with indication, where appropriate of the relevant passages [12] | Relevant to Claim No [13] |
|---|---|---|
| A | Derwent Abstract Accession no. 90-143138/19, Classes B04 and D16, JP,A, 02-088598 (SOMA G) 28 March 1990 (28.03.90) | 1-14 |
| A | Derwent Abstract Accession no. 91-152432/21, Classes B04 and D16, JP,A, 03-087196 (TEIJIN K K) 11 April 1991 (11.04.91) | 1-14 |
| A | Derwent Abstract Accession no. 91-145993/20, Classes B04 and D16, JP,A, 03-083587 (TEIJIN K K) 9 April 1991 (09.04.91) | 1-14 |
| A | Derwent Abstract Accession no. 91-145992/20, Classes B04 and D16, JP,A, 03-083586 (TEIJIN K K) 9 April 1991 (09.04.91) | 1-14 |

*   Special categories of cited documents : [10]

"A"  Document defining the general state of the art which is not considered to be of particular relevance

"E"  earlier document but published on or after the international filing date

"L"  document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O"  document referring to an oral disclosure, use, exhibition or other means

"P"  document published prior t the international filing date but later than the priority date claimed

"T"  Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"  document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y"  document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"  document member of the same patent family

## IV.    CERTIFICATION

| Date of the Actual Completion of the International Search<br>28 August 1992 (28.08.92) | Date of Mailing of this International Search Report<br>- 7 SEP 1992  (07·09·92) |
|---|---|
| International Searching Authority<br><br>**AUSTRALIAN PATENT OFFICE** | Signature of Authorized Officer<br><br>A BESTOW |

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

**V.** ☐ **OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE** [1]

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4a

**VI.** ☐ **OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING** [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest
☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.